Name of Procedure:

Polarized Light Microscopy

Suggested Uses:

The polarized light microscope serves as a tool for the testing of excipients, the use of a microcrystalline test for the testing of drugs and for the determination of optical isomers.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized light microscope Microscope slides Objective centering screws Spatula Specimen

Kohler Illumination for Microscopes With a Built-in Illuminator:

- 1. Focus on any slide preparation of small dry particles using the coarse and fine adjustment knobs.
- 2. Adjust the observation tube.
 - 1. For one ocular tube:
 - a. Focus specimen using the fine adjustment knob.
 - b. Focus ocular cross hairs by rotating the ocular top lens.
 - 2. For binocular tubes:
 - a. Adjust the interpupillary distance by sliding the knurled dovetail slides of the right and left eyepiece tubes, until perfect binocular vision is obtained.
 - b. Looking through the right eyepiece (with cross hairs in view) with your right eye, rotate the upper helicoid ring of the eyepiece until the cross hairs are sharply focused.
 - c. Focus on the specimen with the coarse and fine adjustment knobs so that the sharp images of the specimen and cross hairs can be obtained simultaneously.
 - d. Now look at the image through the left eyepiece with your left eye and rotate the diopter adjustment ring to focus on the specimen without using the coarse and fine adjustment knobs.

Kohler Illumination for Microscopes With a Built-in Illuminator (continued):

- 3. Center the stage rotation (with the objective centering screws) for the highest dry objective so the specimen rotates evenly about the x and y axis of the cross hairs.
- 4. Close the field diaphragm.
- 5. Make sure the image of the field diaphragm is in the center of view by adjusting the condenser using the attached condenser centering screws.
- 6. Focus the image of the field diaphragm by turning the substage condenser knob up or down.
- 7. Repeat Step 5 if necessary.
- 8. Open the field diaphragm to the edge of the field of view.
- 9. If the microscope has an adjustable lamp do the following Steps 1-4, if not, then go to Step 10.
 - 1. Introduce the Bertrand lens to check the focus and centration of the filament, and remove the ocular and observe the objective back focal plane.
 - 2. Focus image of the filament in the objective back focal plane (adjustment on lamp).
 - 3. Center image of the filament (adjustment on lamp).
 - 4. Turn Bertrand lens out.
- 10. Swing in the next highest objective and center it with the objective centering screws on that objective mount. Repeat this step for any remaining objectives.
- 11. Adjust the substage aperture diaphragm for optimum contrast and adjust the field diaphragm image size to the edge of the field of view for each objective.

Operation of the Polarized Light Microscope:

- 1. Switch on the light source.
- 2. Place the specimen slide on the stage.
- 3. Adjust the desired light intensity with the control lever.
- 4. Make sure field diaphragm is open to the edge of the field view.
- 5 Focus with coarse and fine adjustments for the desired objective.
- 6. Move the microscope slide around to view the entire specimen, adjusting the focus accordingly.
- 7. If the objective is changed:
 - 1. Adjust the fine focus adjustment.
 - 2. Set the field diaphragm to just inside the field of view.
 - 3. Adjust the aperture diaphragm for optimum contrast and resolution.

Literature References:

"Particle Characterization by PLM, Part I No. Polarities," <u>Microscope</u>, 30:3 185-196 (1982).

Instruction Manual for the Olympus Microscope, Model BHSP

Name of Procedure:

Polarized Light Microscopy 5% Mercuric Chloride with optional 0.05N Hydrochloric Acid Solution

Suggested Uses:

Microcrystalline test for heroin and caffeine.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Weighing paper Graduated cylinder Glass stirring rod Glass beaker Reagent bottle Mercuric chloride Distilled water Concentrated hydrochloric acid

Formula for Preparing Reagent:

For 5% mercuric chloride reagent:

- 1. Weigh out 1.5 grams of mercuric chloride.
- 2. Mix the mercuric chloride with 30 milliliters of distilled water (giving 5% W.V.) .
- 3. Pour prepared solution in a reagent bottle.
- 4. Properly label reagent bottle.

Formula for Preparing Reagent (continued):

For 0.05N Hydrochloric Acid solution:

- 1. Measure out 1 milliliter of concentrated hydrochloric acid.
- 2. Mix the hydrochloric acid with 250 milliliters of distilled water.
- 3. Pour prepared solution in a reagent bottle.
- 4. Properly label reagent bottle.

Quality Control Check:

Check the reagents with a known standard of heroin using the application procedure listed below.

Expiration Date of Chemical Reagent:

The reagents can be used until depletion provided they are stored in airtight reagent bottles.

Application of Procedure on Evidence:

Heroin: A small sample portion of the sample is placed on a microscope slide and a edge of the drop under non-polarized and/or polarized light.

- **Note:** The test can also be done by mixing the sample with a drop of dilute hydrochloric acid first and then adding the mercuric chloride reagent.
- **Caffeine:** A small portion of the sample is placed on a microscope slide and a drop of mixture. The crystals are observed under non-polarized and/or polarized

Record Results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Always dispose of used microscope slides in a broken glass container.

Literature References:

Butler, William P., <u>Methods of Analysis</u>, Internal Revenue Service, Publication No. 341 (Rev. 6-67).

Name of Procedure:

Polarized Light Microscopy Wagenaar Reagent

Suggested Uses:

Microcrystalline test for barbiturates.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Weighing paper Graduated cylinder Glass stirring rod Glass beaker Reagent bottle Cupric sulfate Distilled water Ethylenediamine

Formula for Preparing Reagent:

- 1. Weigh out 1.5 grams of cupric sulfate.
- 2. Place the 1.5 grams of cupric sulfate in a beaker.
- 3. Measure out 30 milliliters of distilled water.
- 4. Add the 30 milliliters of water to the 1.5 grams of cupric sulfate and stir until dissolved.
- 5. Add ethylenediamine to the 5% cupric sulfate solution until the solution becomes dark violet.
- 6. Pour prepared solution in a reagent bottle.
- 7. Properly label reagent bottle.

Quality Control Check:

Check the reagent with a known barbiturate standard using the application procedure listed below.

Expiration Date of Chemical Reagent:

The reagent can be used until depletion provided it is stored in an airtight reagent bottle.

Application of Procedure on Evidence:

- 1. Place a small portion of the crushed substance on the microscope slide.
- 2. Place a drop of the Wagenaar's reagent on the microscope slide so that it is in contact with the edge of the sample.
- 3. View the crystal formation using the polarized light microscope.
- 4. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Always dispose of used microscope slides in a broken glass container.

Literature References:

Butler, William P., <u>Methods of Analysis</u>, Internal Revenue Service, Publication No. 341 (Rev. 6-67).

Name of Procedure:

Polarized Light Microscopy Gold Chloride in 20% Acetic Acid with optional 0.05N Hydrochloric Acid Solution

Suggested Uses:

Microcrystalline test for cocaine and phencyclidine (PCP).

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Weighing paper Graduated cylinder Glass stirring rod Glass beaker Reagent bottle Glacial acetic acid Distilled water Gold chloride Concentrated hydrochloric acid

Formula for Preparing Reagent:

For Gold Chloride in 20% Acetic Acid:

- 1. Measure out 40 milliliters of water in a graduated cylinder.
- 2. Add glacial acetic acid to the 40 milliliters of water in the graduated cylinder and bring to a total volume of 50 milliliters to make a 20% acetic acid solution.
- 3. Add the 1.0 gram of gold chloride from the ampule to the 20% acetic acid solution.
- 4. Stir until dissolved.
- 5. Pour prepared solution in reagent bottle.
- 6. Properly label reagent bottle.

For 0.05N Hydrochloric Acid solution:

- 1. Measure out 250 milliliters of water and place in a beaker.
- 2. Measure out 1.0 milliliter of concentrated hydrochloric acid and combine it with the 250 milliliters of water.
- 3. Pour solution into a reagent bottle.
- 4. Properly label reagent bottle.

Quality Control Check:

Check the reagents with a known standard of cocaine using the application procedure listed below.

Expiration Date of Chemical Reagent:

The reagent can be used until depletion provided they are stored in airtight reagent bottles.

Application of Procedure on Evidence:

- 1. Place a small portion of crushed substance on a microscope slide.
- 2. Place one drop of 0.05N hydrochloric acid solution on the substance on the microscope slide and mix them together (**Optional Step**).

Application of Procedure on Evidence (continued):

- 3. Place one drop of gold chloride/20% acetic acid reagent on the substance.
- 4. View the crystal formation using a polarized light microscope.
- 5. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Hydrochloric acid is a strong oxidizing agent and corrosive.

Always dispose of used microscope slides in a broken glass container.

Literature References:

Bureau of Narcotics and Dangerous Drugs Seminar, 1970.

Moore, Richard A., and Stanley P. Souse, <u>Analytical Manual</u>, Laboratory Division, Bureau of Narcotics and Dangerous Drugs, United States Department of Justice.

Name of Procedure:

Polarized Light Microscopy Free Acid using 2% Sodium Hydroxide and 5% Sulfuric Acid Solutions

Suggested Uses:

Microcrystalline test for barbiturates.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Weighing paper Graduated cylinder Glass stirring rod Glass pipet and bulb Reagent bottles Sodium hydroxide Distilled water Concentrated sulfuric acid

Formula for Preparing Reagent:

For 2% Sodium Hydroxide solution:

- 1. Weigh out 0.5 gram of sodium hydroxide and place in a graduated cylinder.
- 2. Add enough distilled water to bring the total volume to 25 milliliters.
- 3. Stir until dissolved.
- 4. Pour solution in a reagent bottle.
- 5. Properly label reagent bottle

For 5% Sulfuric Acid solution:

- 1. Measure out 95 milliliters distilled water in a graduated cylinder.
- 2. Add concentrated sulfuric acid to the graduated cylinder to read a total volume of 100 milliliters.

Formula for Preparing Reagent (continued):

- 3. Pour solution into reagent bottle.
- 4. Properly label reagent bottle.

Quality Control Check:

Check the reagent with a known barbiturate standard using the application procedure listed below.

Expiration Date of Chemical Reagent:

The reagents can be used until depletion provided they are stored in airtight reagent bottles.

Application of Procedure on Evidence:

- 1. Place a small portion of the crushed substance on a microscope slide.
- 2. Place a drop of the 2% sodium hydroxide solution on the substance.
- 3. Place a drop of the 5% sulfuric acid solution on the dissolved substance.
- 4. View the crystal formation using the polarized light microscope.
- 5. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Always dispose of used microscope slides in a broken glass container.

Sulfuric acid is a strong oxidizing agent and corrosive.

Sodium hydroxide is caustic.

Literature References:

Developed by Chemist J.R. Daniel of the North Carolina State Bureau of Investigation

Drug Chemistry Laboratory, in use in the laboratory since 1975. **Name of Procedure:**

Polarized Light Microscopy 0.05N Hydrochloric Acid Reagent

Suggested Uses:

Microcrystalline test for excipients and diluents.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Graduated cylinder Glass stirring rod Glass beaker Reagent bottle Concentrated hydrochloric acid Distilled water

Quality Control Check:

Check the reagent with a known excipient or diluent standard using the application procedure listed below.

Formula for Preparing Reagent:

- 1. Measure out 250 milliliters of water and place in a beaker.
- 2. Measure out 1.0 milliliter of concentrated hydrochloric acid and combine it with the 250 milliliters of water.
- 3. Pour solution into a reagent bottle.
- 4. Properly label reagent bottle.

Expiration Date of Chemical Reagent:

The reagent can be used until depletion provided it is stored in an airtight reagent bottle.

Application of Procedure on Evidence:

- 1. Place a small portion of the crushed substance on a microscope slide.
- 2. Place one drop of the 0.05N hydrochloric acid on the substance.
- 3. Immediately view specimen for any effervescence, solubility characteristics, crystal shape(s) and color(s) using the crossed and/or uncrossed polars of the polarized light microscope.
- 4. **Option:** Steps 2 and 3 may be omitted, viewing specimen dry.
- 5. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Always dispose of used microscope slides in a broken glass container.

Hydrochloric acid is a strong oxidizing agent and corrosive.

Literature References:

Developed by Chemist J.R. Daniel of the North Carolina State Bureau of Investigation Drug Chemistry Laboratory, in use in the laboratory since 1975.

Name of Procedure:

Polarized Light Microscopy Methanol

Suggested Uses:

Microcrystalline test for excipients and diluents.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Reagent bottle Methanol

Formula for Preparing Reagent:

- 1. Fill reagent bottle with methanol.
- 2. Properly label bottle.

Expiration Date of Chemical Reagent:

The reagent can be used until depletion provided it is stored in an airtight reagent bottle.

Application of Procedure on Evidence:

- 1. Place a small portion of the substance on the microscope slide.
- 2. Place one drop of methanol on the substance.
- 3. Immediately view specimen for solubility characteristics, crystal shape(s) and color(s), using the crossed and/or uncrossed polars of the polarized light microscope.
- 4. **Option:** Steps 2 and 3 may be omitted, viewing the specimen dry.
- 5. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Always dispose of used microscope slides in a broken glass container.

Literature References:

Developed by Chemist J.R. Daniel of the North Carolina State Bureau of Investigation Drug Chemistry Laboratory, in use in the laboratory since 1975.

Name of Procedure:

Polarized Light Microscopy Distilled Water

Suggested Uses:

Microcrystalline test for excipients and diluents.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Eye protection Laboratory coat Spatula Microscope slides Reagent bottle Distilled water

Formula for Preparing Reagent:

- 1. Fill reagent bottle with distilled water.
- 2. Properly label bottle.

Expiration Date of Chemical Reagent:

The reagent can be used until depletion.

Application of Procedure on Evidence:

- 1. Place a small portion of the substance on a microscope slide.
- 2. Place one drop of distilled water on the substance.
- 3. Immediately view specimen polars for solubility characteristics, crystal shape(s) and color(s), using the crossed and/or uncrossed polars of the polarized light microscope.
- 4. **Option:** Steps 2 and 3 may be omitted, viewing the specimen dry.
- 5. Record results.

Safety Concerns:

Eye protection and a laboratory coat should be worn when using this reagent for the micro crystalline test.

Always dispose of used microscope slides in a broken glass container.

Literature References:

Developed by Chemist J.R. Daniel of the North Carolina State Bureau of Investigation Drug Chemistry Laboratory, in use in the laboratory since 1975.

Name of Procedure:

Polarized Light Microscopy Volatility Test using Gold Chloride in water with Concentrated Sodium Hydroxide Solution

Suggested Uses:

Microcrystalline test for 3,4-methylenedioxyamphetamine (MDA), amphetamine, and methamphetamine.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Weighing paper Graduated cylinder Glass stirring rod Glass beaker Reagent bottles **Distilled water** Sodium hydroxide Gold chloride Spot plate

Formula for Preparing Reagent:

For gold chloride in water:

- 1. Measure out 20 milliliters distilled water and place in a beaker.
- 2. Add 1.0 gram gold chloride.
- 3. Stir until dissolved.
- 4. Pour solution into a reagent bottle.
- 5. Properly label reagent bottle.

Formula for Preparing Reagent (continued):

For concentrated Sodium Hydroxide solution:

- 1. Measure out 20 milliliters of water and pour into beaker
- 2. Add enough sodium hydroxide to obtain saturation (no more sodium hydroxide will dissolve in solution).
- 3. Pour solution in a reagent bottle.
- 4. Properly label reagent bottle.

Quality Control Check:

Check the reagents with a known standard of methamphetamine using the application procedure listed below.

Expiration Date of Chemical Reagent:

The reagent can be used until depletion provided it is stored in an airtight reagent bottle.

Application of Procedure on Evidence:

- 1. Place a small portion of the substance in a clean well of the spot plate.
- 2. Add 1 drop of the concentrated sodium hydroxide solution to the well and stir briefly.
- 3. Place one drop of the gold chloride/water reagent on a microscope side.
- 4. Invert the slide over the well with the concentrated sodium hydroxide solution.
- 5. Let stand for a few minutes.
- 6. Reinvert the slide and view any crystal formation using the polarized light microscope.
- 7. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the micro crystalline test.

Safety Concerns (continued):

Always dispose of used microscope slides in a broken glass container.

Sodium hydroxide is caustic.

Literature References:

Chichilo, Peter, Paul A. Clifford, William Herwitz and Helen Reynolds, <u>Official Methods</u> <u>of the Association of Official Agriculture Chemist</u>, 10th edition, Washington: AOAC, 1965.

Moore, Richard A., and Stanley P. Sobol, <u>Analytical Manual</u>. Laboratory Division Bureau of Narcotics and Dangerous Drugs, United States Department of Justice.

Name of Procedure:

Polarized Light Microscopy Volatility test using Gold Chloride in dilute Phosphoric Acid with 10% Sodium Hydroxide

Suggested Uses:

Microcrystalline test for d-(or l-), and dl-amphetamine, and d-(or l-), and dl-methamphetamine.

Apparatus Needed to Perform Procedure Including Preparation of Reagent:

Polarized Light Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope sides Weighing paper Graduated cylinder Glass stirring rods Glass beakers Reagent bottles Gold chloride 1.0 gram ampule Concentrated phosphoric acid **Distilled water** Spot plate Sodium hydroxide d-amphetamine standard I-amphetamine standard d-methamphetamine standard I-methamphetamine standard

Formula for Preparing Reagent:

For gold chloride in phosphoric acid:

- 1. Measure out 20 milliliters of distilled water.
- 2. Measure out 10 milliliters of concentrated phosphoric acid.

Formula for Preparing Reagent (continued):

- 3. Pour the 10 milliliters of acid into the water and stir.
- 4. Pour 20 milliliters of the diluted phosphoric acid solution into a beaker.
- 5. Add 1.0 gram of gold chloride to the 20 milliliter solution in the beaker.
- 6. Stir until dissolved.
- 7. Pour in a reagent bottle.
- 8. Properly label reagent bottle.

For 10% Sodium Hydroxide:

- 1. Weigh out 2.5 grams of sodium hydroxide and place in a graduated cylinder.
- 2. Add enough distilled water to bring the total volume to 25 milliliters.
- 3. Stir until dissolved.
- 4. Pour the solution into a reagent bottle.
- 5. Properly label reagent bottle.

Quality Control Check:

Check the reagents with a known standard of d-methamphetamine using the application procedure listed below.

Expiration Date of Chemical Reagent:

The reagents can be used until depletion provided they are stored in airtight reagent bottles.

Application of Procedure on Evidence:

For unknown sample only:

- 1. Place a small portion of the unknown substance in a clean well of the spot plate.
- 2. Add 1 drop of the 10% sodium hydroxide solution to the well and stir briefly.
- 3. Place 1 drop of the gold chloride/phosphoric acid solution on a microscope slide.
- 4. Invert the slide over the well with the 10% sodium hydroxide solution.
- 5. Let stand for a few minutes.
- 6. Reinvert the slide and view any crystal formation using the polarized light microscope.
- 7. Record results.

Application of Procedure on Evidence (continued):

For unknown sample mixed with the known d- or l- isomer of the suspected compound:

- 1. Take a small portion of the unknown and mix thoroughly with an equal portion of the d- or l- isomer of the known compound.
- 2. Place a small portion of the mixture in a clean well of the spot plate.
- 3. Add 1 drop of the 10% sodium hydroxide solution to the well and stir briefly.
- 4. Place 1 drop of the gold chloride/phosphoric acid solution on a microscope slide.
- 5. Invert the slide over the well with the 10% sodium hydroxide solution.
- 6. Let stand for a few minutes.
- 7. Reinvert slide and view any crystal formation using the polarizing light microscope.
- 8. If the crystal pattern that forms is different from the crystal pattern of just the unknown and is also identical with the known dl-racemic mixture, then the optical isomer of the unknown substance will be opposite the optical isomer of the known compound used.
- 9. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Always dispose of used microscope slides in a broken glass container.

Sodium hydroxide is caustic.

Phosphoric acid is a strong oxidizing agent and it is corrosive.

Literature References:

Fulton, Charles C., <u>Modern Microcrystalline Test for Drugs</u>, New York: Wiley-Interscience a Division of John Wiley & Sons, 1969.

Chichilo, Peter, Paul A. Clifford, William Herwitz, and Helen Reynolds, <u>Official</u> <u>Methods of Analysis of the Association of Official Agriculture Chemist</u>, 10th edition, Washington: AOAC, 1965.

Literature References (continued):

Teer, Charles B., "Modification of the Microcrystalline Test for d-Amphetamine," <u>BNDD Laboratory Notes</u>, June 8, 1970, No. 5.

Developed for use with amphetamine and methamphetamine by Chemist J.R. Daniel of the North Carolina State Bureau of Investigation, in use in the laboratory since 1980.

Name of Procedure:

Polarized Light Microscopy 50% Acetic Acid and Gold Chloride in 50% Acetic Acid

Suggested Uses:

Microcrystalline test for determining the optical isomer of propoxyphene.

Apparatus Needed To Perform Procedure Including Preparation of Reagent:

Polarized Microscope Fume hood Gloves Eye protection Laboratory coat Spatula Microscope slides Weighing paper Graduated cylinder Glass stirring rod Glass beaker Reagent bottles Distilled water

Formula for Preparing Reagent:

For 50% Acetic Acid:

- 1. Measure out 20 milliliters of distilled water in a graduated cylinder.
- 2. Add glacial acetic acid to the graduated cylinder until a total volume of 40 milliliters is obtained.
- 3. Pour solution into a reagent bottle.
- 4. Properly label reagent bottle.

For Gold Chloride in 50% Acetic Acid:

- 1. Prepare a second 50% acetic acid solution as above (Steps 1 and 2).
- 2. Mix the contents of a 1 gram ampule of gold chloride into the 50% acetic acid solution.
- 3. Pour solution into a reagent bottle.
- 4. Properly label reagent bottle.

Quality Control Check:

Check the reagents with a known standard of d-propoxyphene using the application procedure listed below.

Expiration Date of Chemical Reagent:

The reagents can be used until depletion provided they are stored in airtight reagent bottles.

Application of Procedure on Evidence:

- 1. Take 2 small samples (approximately 0.1 milligram) of the unknown and place them near each end of a microscope slide.
- 2. Take a sample of the known standard (approximately 0.1 milligram) and mix with one of the unknown samples on the microscope slide.
- 3. Place a drop of reagent #1 on each sample on the slide.
- 4. Place a drop of reagent #2 on each sample on the slide.
- 5. Observe the resulting mixtures with uncrossed and crossed polars for crystal formation.
- 6. Record results.

Safety Concerns:

Always wear eye protection, gloves, and a laboratory coat when preparing this reagent.

Eye protection and a laboratory coat should be worn when using this reagent for the microcrystalline test.

Always dispose of used microscope slides in a broken glass container.

Literature References:

Developed by Chemist J.R. Daniel of the North Carolina State Bureau of Investigation Drug Chemistry Laboratory, in use in the laboratory since 1975.