OSAC 2022-S-0023
Standard Guide for the Forensic Analysis of Explosives by Polarized Light Microscopy

Ignitable Liquids, Explosives, and Gunshot Residue Subcommittee
Chemistry: Trace Evidence Scientific Area Committee (SAC)
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

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Prepared by
Ignitable Liquids, Explosives, and Gunshot Residue Subcommittee
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1. Scope

1.1 This standard addresses the use of polarized light microscopy (PLM) to identify explosive-related compounds from intact explosives and post-blast residues containing unconsumed explosive compounds or their solid reaction products, and to isolate them for further analysis.

1.2 This standard is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (see Practice E2917), and demonstrated proficiency to perform forensic casework (refer to the T/SWGFEX Suggested Guide for Explosives Analysis Training).

1.3 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2. Referenced Documents

2.1 ASTM Standards

E620 Practice for Reporting Opinions of Scientific or Technical Experts
E860 Practice for Examining and Preparing Items that are or may become Involved in Criminal or Civil Litigation
E1732 Terminology Related to Forensic Science
E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Program
E3196 Terminology Relating to the Examination of Explosives
E3253 Practice for Establishing an Examination Scheme for Intact Explosives
E3329 Practice for Establishing an Examination Scheme for Explosive Residues

2.2 Other Resources

Technical/Scientific Working Group for Fire and Explosion Analysis (T/SWGFEX) Suggested Guide for Explosive Analysis Training (https://www.nist.gov/system/files/documents/2018/09/21/twgfex_suggest_guide_for_explosive_analysis_training.pdf)

3. Terminology

3.1 Definitions - For definitions of terms used in this guide other than those listed in 3.2, see E1732 and E3196.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 alpha (α) - symbol representing the lowest of the three principal refractive indices of a biaxial crystal.

3.2.2 anomalous interference colors, n – atypical polarization colors which occur due to variation with wavelength of the refractive index (very few substances display this characteristic).

3.2.3 Becke line, n – a halo observed near the boundary of a transparent particle that is mounted in a medium that differs from the particle's refractive index.

3.2.4 Becke line method, n - method for determining the refractive index of a transparent particle relative to its mountant by noting the direction in which the Becke line moves when the focus is changed.

Discussion—The Becke line always moves toward the higher refractive index medium (particle or mountant) then the focus is raised, and towards the lower refractive index medium when the focus is lowered. At the point where the index of the particle matches the index of the mounting medium, the Becke line is no longer visible. The Becke line is generally viewed at a wavelength of 589 nm (the D line of sodium [n_D]).

3.2.5 beta (β) - symbol representing the intermediate principal refractive index of a biaxial crystal.

3.2.6 biaxial, adj - an anisotropic crystal in the orthorhombic, monoclinic, or triclinic system with three principal refractive index directions (α, β, γ) and two optic axes that are isotropic.

3.2.7 birefringence, n - the numerical difference between the maximum and minimum refractive indices of anisotropic substances.

3.2.8 crystal system, n - crystals are classified according to their crystallographic axes length and the angles between them. There are six crystal systems: cubic, tetragonal, hexagonal, orthorhombic, monoclinic, and triclinic. All crystal systems are anisotropic except for the cubic system, which is isotropic.

3.2.9 epsilon (ε) - any vibration direction in the plane of the c axis for uniaxial crystals.

3.2.10 euhedral, adj - well-formed crystals bounded by plane faces.

3.2.11 extinction, n - condition when an anisotropic substance appears dark when observed between crossed polarizers and occurs when the vibration directions of the specimen are parallel to the vibration directions of the polarizer and analyzer.

3.2.12 extinction, oblique, n - a type of extinction in which the vibration directions are inclined at an angle relative to a crystal face.

3.2.13 extinction, parallel, n - a type of extinction in which the vibration directions are parallel to the crystal faces.

3.2.14 extinction, symmetrical, n - a type of extinction in which the vibration directions bisect a prominent interfacial angle of the crystal

3.2.15 gamma (γ) - symbol representing the highest of the three principal refractive indices of a biaxial crystal.

3.2.16 interference figure, n – pattern observed during conoscopic observation of an anisotropic material which consists of a combination of extinction positions and interference colors corresponding to the full cone of directions by which the sample is illuminated.

Discussion—Conoscopic observations are typically made by inserting a Bertrand lens into the body tube or by removing an eyepiece and viewing down the body tube.

3.2.17 fusion methods, *n* - A process in which compounds are heated on a microscope slide and observed via PLM during heating, melting, and, upon cooling, recrystallization of the melt.

Discussion—Fusion methods frequently employ a temperature controlled hot stage capable of at least 300^{O} Celsius placed on the stage of a polarizing light microscope.

3.2.18 omega (ω) - any vibration direction in the plane of the a axis for uniaxial crystals.

3.2.19 optic axial angle (2V), n - the acute angle between two optic axes of a biaxial crystal.

3.2 20 optic axis, n - a direction of isotropic light propagation in an anisotropic crystal. Uniaxial crystals have one optic axis; biaxial crystals have two optic axes.

3.2.21 optic sign, n - determined by the relationship of the refractive indices of a material. For uniaxial crystals, if $\varepsilon > \omega$, the crystal is positive (+); if $\omega > \varepsilon$, the crystal is negative (-). For biaxial crystals, if $\gamma-\beta > \beta-\alpha$, the crystal is positive (+); if $\gamma-\beta < \beta-\alpha$, the crystal is negative (-).

3.2 22 polymorphism, n - crystallization into two or more chemically identical but crystallographically distinct forms.

3.2.23 relief, n - contrast between a particle or crystal and its media due to the difference between their refractive indices. The greater the numerical difference in refractive indices, the greater the relief.

3.2.24 subhedral, adj - crystals with imperfectly developed faces.

3.2.25 uniaxial, adj - an anisotropic crystal in the tetragonal or hexagonal system having one optic axis (isotropic direction) and either two (tetragonal) or three (hexagonal) directions which are alike and perpendicular to the direction of the optic axis.

4. Summary of Practice

4.1 Solid chemical components in samples are analyzed using a polarized light microscope (PLM). The optical crystallographic properties such as refractive index, birefringence, and morphology are determined. Determination of these properties, which are different for various explosive components and other chemical compounds, can aid in the identification of explosives and explosive residues submitted to the forensic laboratory.

4.2 Optical crystallographic properties of an unknown component can be determined by either mounting the component in a refractive index liquid, recrystallizing the component from a small drop of solvent, or recrystallizing the component from its melt, i.e., recrystallization occurring when a melted compound is cooled. Recrystallized components can be isolated for further analysis using other analytical techniques such as Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, or scanning electron microscopy-energy dispersive x-ray spectroscopy (SEM-EDS).

4.3 Water-soluble components, such as inorganic salts used as oxidizers, are dissolved in a drop of water and tested using reagents to form characteristic microcrystals. Microcrystal tests can aid in the determination of anions and cations present in oxidizers and other components of explosives.

4.4 The optical crystallographic properties of an inorganic compound and microcrystal testing for its ions are independent techniques that provide complementary information. A combination of tests that includes the optical crystallographic characterization of a compound and microchemical tests to confirm the anion and cation are used to identify oxidizers and other salts present in explosives.

4.5 A combination of tests that include optical crystallographic characterization or microchemical tests in conjunction with other independent techniques are used to identify chemical components present in explosives. Refer to ASTM E3253 and E3329 for the requirements for the identification of commonly encountered explosives and chemical components present in explosive materials.

5. Significance and Use

5.1 This standard is designed to assist the analyst using polarized light microscopy for the examination of test samples for the presence of explosives.

5.2 Intact explosives and post-blast residues that contain unconsumed explosive components or their solid reaction products are suitable for this standard (E3253, E3329). Particles only a fraction of a millimeter in size can be examined using this standard.

5.3 This standard is not suitable for the examination of smokeless powder or liquid explosives.

5.4 Some of the microscopical techniques described in this standard allow for the recovery of the specimen for subsequent analysis, but others, such as microcrystal tests, are destructive.

5.5 Identifications of explosive-related components based on these properties shall be part of an analytical scheme as described in ASTM E3253 and E3229.

6. Apparatus

6.1 Polarized light microscope - A compound microscope with a rotating stage, polarizing filters, substage condenser, and compensator slot.

Note 1—Most commercially available polarized light microscopes should be adequate for the forensic analysis of explosives and appropriate for use following this standard.

6.2 Objectives - Strain-free typically 10X, 20X, and 40X.
6.2.1 A 40X objective with a numerical aperture of 0.65 or higher is recommended to provide the widest view of the back focal plane for conoscopic observations.
6.2.2 A 10X dispersion staining objective is required if performing dispersion staining.

6.3 Compensator - A full wave compensator for use with the microscope. Optional compensators include quarter-wave plate and quartz wedge.

6.4 Hot stage - A temperature controlled hot stage for use with the microscope with a range of approximately 30°C to 300°C.

6.5 Alcohol lamp

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. It is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *10% platinum chloride* (H_2PtCl_6) in water

7.3 *zinc uranyl acetate*

7.4 *ammonium molybdate* ($(NH4)_6Mo_7O_{24}.4H2O$)

7.5 *squaric acid*

7.6 *potassium iodide* (KI)

7.7 *nitron sulfate*

7.8 *strychnine sulfate*

7.9 *0.3% methylene blue* in deionized water (Methylene Blue Reagent)

7.10 *20% potassium nitrate* (KNO_3) in deionized water

7.11 *saturated zinc sulfate* ($ZnSO_4$) in deionized water

7.12 Toluene or methylene chloride

7.13 Sodium hydroxide

7.14 Deionized water

7.15 Reference materials, including reagent grade chemicals and explosive materials, can be obtained from commercial and retail sources or directly from the distributor or manufacturer.

7.16 Refractive index liquids with known temperature coefficients (*dn/dt*) and tolerance at n_D

7.17 Microscope slides and coverslips

7.18 Glass rod with a tapering tip of approximately 1 mm or less in diameter

7.19 Wood toothpicks

7.20 Glass ring with an approximate diameter of 10-12 mm and 5-10 mm in height

7.219 Tungsten needles

7.22 589 nm light filter

8. Initial Microscopical Examination

8.1. Preliminary assessment of the samples using visual and stereo microscopical examinations are conducted following E3253-21 and E3329-21. If preliminary examinations indicate that the sample is an explosive mixture or a component thereof, a portion of the sample is examined by PLM in order to obtain further information regarding the composition of the sample.

8.2 One to three particles or grains (approximately 1 mg or less) are mounted on a glass microscope slide with a cover slip and dispersed in a refractive index oil. For post-blast evidence a similar quantity of residue material is mounted in the same manner. Any refractive index oil in the 1.400 to 1.800 range should be used, however, if preliminary examinations indicate a particular explosive material, e.g., black powder, ammonium nitrate-fuel oil (ANFO) or Composition C-4, the examiner should select a refractive index oil that will enable confirmation of characteristic optical crystallographic data based upon Tables 1 and 2.

8.3 The mounted sample is examined with a polarized light microscope at low magnification (e.g. 10X). The magnification is increased as needed to better view particles for examination.

8.4 Charcoal, if present, will appear as irregularly shaped, black and opaque particles, and as brown flakes often with cellular features such as pits or cell walls (**12**).

8.5 Most common explosives and oxidizers are colorless when viewed microscopically with transmitted plane polarized light. Crossed polarizers are used to determine if the crystals are isotropic or anisotropic. Interference colors are assessed to determine if the birefringence is low (less than ~0.01), moderate (~0.01-0.10) or high (above ~0.10).

8.6 The relative refractive indices of the crystals present in the specimen are determined using the Becke line method or dispersion staining.

8.7 The morphology, relative refractive indices, and interference colors can indicate the presence of an explosive ingredient or chemical component. See Tables 1 and 2.

8.7.1 If explosive related components are indicated, additional microscopical analysis is performed to further characterize the components via optical crystallography (Sections 9 & 10).

8.7.2 If inorganic salts are indicated, additional microscopical analysis may be performed by microcrystal tests (Section 11).

8.7.3 If no explosive related components are indicated, further analysis using microscopy or other instrumental analyses could be performed to characterize or identify the substance in the case sample. Such examinations are beyond the scope of this document.

9. Optical Crystallography by Solvent Recrystallization

9.1 Solvent recrystallization allows for a more detailed optical crystallographic characterization of a compound as it recrystallizes from a small drop of suitable solvent.

9.2 Water recrystallization for inorganic oxidizers and salts

9.2.1 A small drop (5-7 mm in diameter) of distilled or deionized water is placed on a microscope slide. 1-3 particles or grains (approximately 1 mg or less) from the sample with similar appearance are added to the drop. If the specimen does not readily dissolve, it should be crushed and dispersed with the tip of a glass rod to aid dissolution.

9.2.1.1 Alternatively, if a large amount of insoluble components are present, a portion of the specimen can be mixed with water and filtered to remove water insoluble components such as carbon, aluminum, and sulfur. A small drop of the filtered water extract is then placed on a microscope slide.

9.2.2 The test drop is examined using the PLM and a low magnification objective, e.g., 10X. Crystals typically form at the edge of the drop first. Distorted or subhedral crystals, if formed, are pushed back into the center of the drop using a device such as a glass rod, toothpick or metal probe to produce characteristic euhedral crystals. Multiple water-soluble components can be present in the sample and, depending on their concentration and solubility, could recrystallize at different times as the drop evaporates.

9.2.3 Crystals formed as the drop evaporates are examined using plane-polarized and crossed-polarized light. Observe their crystal habit, relief, and whether they are isotropic or anisotropic.

9.2.4 For anisotropic euhedral crystals, extinction is determined to be parallel, symmetrical, or oblique.

9.2.5 Interference colors are assessed to determine if the birefringence is low (less than ~0.01), moderate (~0.01-0.10), or high (above ~0.10).

9.2.6 Observable interference figures are used to determine if the crystal is uniaxial or biaxial and the optic sign. Interference figures can also be used to relate morphology of the crystal to its optical orientation, and, if the crystal is biaxial, estimate the optic axial angle (2V).

9.2.7 The optical crystallographic properties of the crystals are documented by description, sketch, or photomicrographs.

9.2.8 The optical crystallographic properties of the crystals formed are compared to recrystallized known reference materials or published references (6, 8). A tentative identification is made if the optical crystallographic properties of the specimen are the same as a given compound. Table 1 lists optical crystallographic properties for twelve common oxidizers.

9.2.9 If two or more oxidizers recrystallize from the drop, their tentative identification is compared to the optical properties determined from examinations of the intact sample (Section 8 and 10) to assess if a metathetical reaction has occurred. If a metathetical reaction occurred, the optical properties of the intact crystals and those obtained from recrystallization will be different.

9.2.10 Depending on the analytical scheme chosen, dried crystals can be 1) mounted in refractive index oils for refractive index determination (Section 10), 2) analyzed via microcrystal tests (Section 11), or 3) analyzed by other instrumental techniques (E3253, E3329) If the crystals cannot be further analyzed on the glass slide, a tungsten needle is used to transfer the crystals from the glass slide to another substrate such as a potassium bromide pellet for additional analysis.

9.3 Organic solvent recrystallization for sulfur

9.3.1 A small drop (5-7 mm in diameter) of a suitable solvent, such as toluene or methylene chloride, is placed on a microscope slide. One to three particles or grains (approximately 1 mg or less) of sample are added to the solvent drop. Sulfur, if present, will recrystallize as highly refractive, birefringent dipyramids (diamond-shaped) or form liquid drops that crystallize when touched with a tungsten needle.

9.3.2 The optical crystallographic properties of the crystals are documented by description, sketch, or photomicrograph.

9.3.3 The optical crystallographic properties of the crystals formed are compared to recrystallized known reference materials or published references (**5**, **6**). A tentative identification is made if the optical crystallographic properties of the specimen are the same as a given compound. Table 1 lists optical crystallographic properties for sulfur.

9.3.4 Depending on the analytical scheme chosen, dried crystals can be further analyzed by other analytical techniques (E3253, E3329). If the crystals cannot be further analyzed while on the glass slide, a tungsten needle is used to transfer crystals from the glass slide to another substrate such as carbon tape for additional analysis.

10. Optical Crystallography Using Refractive Index Liquids

10.1 Organic and inorganic compounds present in explosives are characterized by their optical crystallographic properties by mounting intact material in a liquid of known refractive index.

10.2 One to three particles or grains (approximately 1 mg or less) of the sample, or dried crystals obtained during recrystallization, as described in Section 9, are mounted in a refractive index oil in the 1.400 to 1.800 range. If previous examinations indicate a particular compound, e.g., potassium nitrate or RDX, the examiner should select a refractive index oil that will provide characteristic optical crystallographic data based on Tables 1 and 2.

10.3 The crystals are examined using PLM to determine the optical crystallographic properties such as refractive indices, birefringence, extinction type (parallel, symmetrical, or oblique), optic sign, and optic axial angle (2V).

10.4 Refractive index can be determined using the Becke line method or dispersion staining. The specimen can be mounted in additional refractive index liquids to confirm one or more refractive indices of the indicated compound.

10.5 The optical crystallographic properties of the specimen are compared to known reference materials or published references (e.g., 5, 6, 8, 12-16, 22). A tentative identification is made if the optical crystallographic properties of the specimen are the same as a given compound. Table 1 lists optical crystallographic properties for three fuels (sulfur, ascorbic acid and sucrose) and twelve common oxidizers. Table 2 lists optical crystallographic properties for four organic high explosives.

Table 1. Optical Crystallographic Properties of Common Fuels & Oxidizers

Compound	Crystal System	Refractive Indices/ Optic Angle	Habit	Comments
Sulfur (S) (**12**)	orthorhombic	1.958 2.038 2.245 $2V = 68^{O}(+)$	dipyramids	High relief in solvent. Larger crystals appear yellowish in color.
Ascorbic Acid (**22**)	monoclinic	1.474 1.595 1.746 $2V = 88^{O}(+)$		High order interference colors
Sucrose (**12**, **22**)	monoclinic	1.540 1.567 1.572 $2V = 48^{O}(-)$		
Sodium chlorate ($NaClO_3$) (**8**)	cubic	1.518	squares, rectangles	
Barium nitrate ($Ba(NO_3)_2$) (**8**)	cubic	1.571	cubes octahedra	
Strontium nitrate ($Sr(NO_3)_2$) (**6**)	cubic	1.586	octahedra	
Lead nitrate ($Pb(NO_3)_2$) (**8**)	cubic	1.781	octahedra cubes	High relief in water

Potassium perchlorate ($KClO_4$) (**8**)	orthorhombic	1.4731 1.4737 1.4769 $2V = 50^{O}(+)$	rectangular prisms, rhombs	Low order interference colors
Ammonium perchlorate (NH_4ClO_4) (**8**)	orthorhombic	1.4818 1.4833 1.4881 $2V = 70^{O}(+)$	six-sided prisms	Low order interference colors. Crystals lying on a {110} face will exhibit an off-centered biaxial interference figure.
Sodium perchlorate ($NaClO_4$) (**8**)	orthorhombic	1.4606 1.4617 1.4730 $2V = 69^{O}(+)$	See $NaClO_4.2H_20$	Sodium chlorate recrystallizes as the hydrate. Optical data is for the anhydrate.
Sodium perchlorate dihydrate ($NaClO_4.2H_2O$) (**8**)	monoclinic		diamonds, rhombs	Low relief in water. Some orientations exhibit oblique extinction. Refractive indices not known.
Potassium chlorate ($KClO_3$) (**8**)	monoclinic	1.415 1.517 1.523, $2V = 28^{O}(-)$	diamond-shaped tablets, often truncated	Moderate order interference colors, symmetrical extinction
Potassium nitrate (KNO_3) (**8**)	hexagonal & orthorhombic	1.3346 1.5056 1.5064 $2V = 7^{O}(-)$	rhombohedra & chevrons early; prisms late	The hexagonal forms with high order interference colors appear first at the edge of the drop. Moving them to the middle of the drop with a glass rod or toothpick causes the stable orthorhombic forms to appear.
Ammonium nitrate (NH_4NO_3) (**8, 9**)	orthorhombic	1.413 1.611 1.637 $2V = 35^{O}(-)$	prisms, as blades & rods	Crystal formation is slow due to the high solubility of ammonium nitrate. The resulting crystals exhibit high order interference colors. As a fusion preparation cools, three to four polymorphs are observed. MP = 169C
Sodium nitrate ($NaNO_3$) (**8**)	hexagonal	1.5874 (ω) 1.3361 (ε)	rhombohedra	High order interference colors and symmetrical extinction.

Table 2. Crystallographic and Optical Properties of High Explosives

Compound	Crystal System	Optical Properties	Habit	Comments
RDX (**13**)	orthorhombic	1.578 1.597 1.602 $2V = 53^{O}(-)$		Exhibits anomalous interference colors and dispersed extinction. MP = 204-206C
HMX (**13**)	monoclinic	1.589 1.594 1.73 $2V = 20^{O}(+)$	dipyramids & pinacoids, rare acicular	Typically exhibits high order interference colors. Oblique extinction
TNT (**13**)	orthorhombic	1.543 1.674 1.717		MP = 81C

		2V = 60 (-)		
PETN (**13**)	tetragonal	ω = 1.556 ε = 1.551		MP = 141.5-142C

11. Microcrystal tests

11.1 Microcrystal tests are chemical precipitation reactions in which the target ion forms an insoluble reaction product with the reagent. The crystals formed are examined using a polarized light microscope. Microcrystal tests are used in conjunction with other independent techniques to identify explosive related compounds.

11.2 Microcrystal tests are used to test for anions and cations of oxidizers and other substances such as aluminum that are present in explosives. Many microcrystal tests are available, those commonly used in explosives analysis are listed in Table 3.

11.3 The presence of additional water-soluble compounds can interfere with, or affect the formation of, microcrystals of the target anion or cation. If this occurs, further isolate the target compound and repeat the microcrystal test or use a different analytical technique.

11.4 Prior to casework, the reagents used for the microcrystal tests are tested for reliability using the appropriate positive and negative controls. The crystals obtained with the positive control are compared to the published references (**1, 4, 7, 17, 19, 20, 21**).

11.5 There are four routine methods for conducting microcrystal tests (**4**). Test drops and reagent drops used for microcrystal tests should be approximately 5-7 mm in diameter or smaller. Most reactions take place within one minute. Test drops with lower concentrations may take longer to occur.

Note 2—The methods described below could have different number designations as found in reference 4.

11.5.1 Method 1

11.5.1.1 A drop of deionized water is placed on a clean microscope slide and one or more particles of the sample is added with stirring, crushing and dispersing as needed to dissolve the water-soluble components. The test drop should be kept small. Alternatively, 1-5 grains or particles of the sample are placed in a spot well plate or test tube and 3-4 drops of deionized water are added. The specimen is stirred with a glass rod to disperse and dissolve any water-soluble components. A test drop of the aqueous solution is placed on a clean microscope slide.

11.5.1.2 Place a drop of reagent near, but not touching, the test drop. Depending on the microcrystal test being conducted some reagent solutions are pre-made and a drop added to the slide. Other reagent drops are created on the slide by placing a drop of water near the test drop and adding 1-5 particles of the reagent to the water droplet.

11.5.1.3 The test drop and reagent drop are drawn together using a clear glass rod or toothpick. The preparation is immediately examined microscopically using a 10X objective. Higher magnification can be used but care must be taken to ensure the objective does not contact the test drop.

11.5.1.4 The preparation is examined for the presence of characteristic microcrystals or the lack of a reaction.

11.5.1.5 The formation of microcrystals corresponding to those obtained with the positive control indicates the presence of that anion or cation.

11.5.2 Method 2
11.5.2.1 Follow 11.5.1 except the reagent drop is added to the middle of the test drop.
11.5.2.2 The preparation is examined microscopically as described in Method 1 for the presence of characteristic microcrystals or the lack of a reaction.
11.5.2.3 The formation of microcrystals corresponding to those obtained with the positive control indicates the presence of that anion or cation.

11.5.3 Method 3
11.5.3.1 Follow 11.5.1 except add reagent particles into the middle of the test drop.
11.5.3.2 The preparation is examined microscopically as described in Method 1 for the presence of characteristic microcrystals or the lack of a reaction.
11.5.3.3 The formation of microcrystals corresponding to those obtained with the positive control indicates the presence of that anion or cation.

11.5.4 Method 4
11.5.4.1. A small drop of deionized water is placed on a clean microscope slide. One or more particles of the sample is added to the drop with stirring, crushing, and dispersing as needed to dissolve the water-soluble components. The test drop should be kept small.
11.5.4.2 A glass ring with an internal diameter of about 10-12 mm is placed around the test drop so the drop is in the center of the ring.
11.5.4.3 A small drop of the prepared reagent is placed on a coverslip.
11.5.4.4 A small drop of the volatilizing solution (e.g., 10% sodium hydroxide) is added to the test drop inside the center of the ring.
11.5.4.5 The coverslip is immediately placed onto the glass ring such that the reagent drop is suspended in the center of the ring above the sample.
11.5.4.6 The slide preparation is placed on the microscope stage ensuring sufficient working distance between the top of the glass ring and the objective.
11.5.4.7 The reagent drop is examined for the presence of microcrystals or the lack of reaction.
11.5.4.8 The formation of microcrystals corresponding to those obtained with the positive control indicates the presence of that anion or cation.

11.6 Table 3 lists twelve tests for anions and cations common to low explosives along with the reagent, literature reference, and method as described in 11.5. Microcrystal tests for additional ions can be found in Chamot & Mason (**4**), Benedetti-Pichler (**21**), Wills (**19**), and Hollifield (**20**).

Table 3. Microcrystal Tests for Ions

Cation/anion tested	Microcrystal test & reference	Method
Ammonium	Chloroplatinic acid (**4**)	4
Potassium	Chloroplatinic acid (**4**)	1, if positive, repeat using Method 4. If potassium present and ammonium absent, Method 4 test will be negative
Sodium	zinc uranyl acetate (**4**)	1
Aluminum	ammonium molybdate (**4**)	2

Magnesium	Squaric acid (**19, 20**)	1
Barium	Squaric acid (**19, 20**)	1
Strontium	Squaric acid (**19, 20**)	1
Lead	Potassium iodide (**4**)	1
Nitrate	Nitron sulfate (1, **4**)	1
Perchlorate	Strychnine sulfate (**4**)	1, 3
Perchlorate	Methylene Blue (**7**)	1

12. Fusion Methods

12.1 Fusion methods (**10**) are techniques that allow for the characterization of crystals by determining the melting point (MP) and observing optical crystallographic properties as the sample recrystallizes from the melt. This method is most useful for organic high explosives (**11, 14, 15, 16**) but can also be applied to some inorganic explosives (**9**). Crystalline compounds examined using fusion methods must first be isolated from mixtures by physical or chemical separation.

12.2 Examination Using Temperature Controlled Hot Stage

12.2.1 The isolated crystalline substance is placed on a microscope slide with a cover slip, or on a cover slip (according to the hot stage manufacturer’s instructions).

12.2.2 The preparation is placed in the hot stage at a temperature of approximately 50C. If a particular explosive compound has been indicated from prior analysis, a temperature approximately 10-20C lower than the expected melting point can be used.

12.2.3 The specimen is brought into the field of view. The temperature of the hot stage is raised while the specimen is viewed using the polarized light microscope. The melting point is recorded. The temperature is slowly lowered while observing the recrystallization of the sample from the melt.

12.2.4 The optical crystallographic properties such as morphology, crystal front shape, polymorphism, interference colors, anomalous interference colors, extinction characteristics, interference figure, and optic sign are observed for each solid state as appropriate.

12.3 Alternatively, the sample is heated on a microscope slide using an alcohol lamp (**9**). This method is especially useful for substances with melting points above 300C, including most inorganic oxidizers. An accurate melting point is not determined, but the sample is observed through the microscope as the slide cools to obtain various optical crystallographic properties, as described in 12.2.4.

12.4 Compare the optical crystallographic properties of the specimen to published reference descriptions (**9-11, 14-16**) or a known reference material.

12.5 Mixed fusions

12.5.1 If an explosive compound is indicated using optical crystallography, microcrystal tests, or other techniques, a mixed fusion using a known reference material of the suspected compound can confirm the identity of the unknown (**9, 10**).

12.5.2 A small amount of isolated crystalline substance from the sample is placed on a slide, under a coverslip, but near the edge of the coverslip. The sample is melted over an alcohol lamp. The melted sample should fill approximately half of the area under the coverslip.

12.5.3 Once the unknown sample has cooled and recrystallized, the known reference material is placed near the opposite edge of the coverslip and melted over an alcohol lamp such that the melted standard flows under the coverslip and contacts the previously cooled unknown specimen. The portion of the unknown specimen that contacts the reference material will also melt during this process.

12.5.4 Using the PLM, the zone of mixing is viewed as the preparation cools and recrystallizes. If the unknown compound is the same as the reference material, the crystals of the unknown will continue to grow through the zone of mixing with no change in crystal growth rate or in appearance. If the unknown compound and reference material are different, a change in the crystal growth rate occurs and the crystals in the zone of mixing usually appear different.

12.5.5 Once cooled, the mixed fusion preparation can be inserted into the hot stage at a temperature approximately 10 degrees lower than the melting point of the reference material. While observing the zone of mixing, slowly raise the temperature (1-3 degrees/minute) of the hot stage to the melting point of the reference material If the two compounds are the same, the melting point will be the same across the zone of mixing.

Note 3—This can only be performed if the sample melts below the upper temperature range of the hot stage.

13. Documentation

13.1 Retain all notes and supporting analytical data used for the identification or tentative identification of explosive related compounds in accordance with E620, E860 and E3255. Examples of such data include photomicrographs and detailed descriptions of microscopical observations.

13.2 Record case notes in sufficient detail such that an independent analyst could understand and evaluate the work performed, independently interpret the data, and form an opinion.

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