

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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Standard Guide for the Forensic Analysis of Explosives by Polarized Light Microscopy

Prepared by
Ignitable Liquids, Explosives, and Gunshot Residue Subcommittee
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*OSAC 2022-S-0023, Standard Guide for the Forensic
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Standard Guide for the Forensic Analysis of Explosives By Polarized Light Microscopy

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.

49
50 3.2.4 Becke line method, n - method for determining the refractive index of a transparent particle relative
51 to its mountant by noting the direction in which the Becke line moves when the focus is changed.
52
53 *Discussion*—The Becke line always moves toward the higher refractive index medium (particle or
54 mountant) then the focus is raised, and towards the lower refractive index medium when the focus is
55 lowered. At the point where the index of the particle matches the index of the mounting medium, the
56 Becke line is no longer visible. The Becke line is generally viewed at a wavelength of 589 nm (the D line
57 of sodium [n_D]).
58
59 3.2.5 beta (β) - symbol representing the intermediate principal refractive index of a biaxial crystal.
60
61 3.2.6 biaxial, adj - an anisotropic crystal in the orthorhombic, monoclinic, or triclinic system with three
62 principal refractive index directions (α , β , γ) and two optic axes that are isotropic.
63
64 3.2.7 birefringence, n - the numerical difference between the maximum and minimum refractive indices
65 of anisotropic substances.
66
67 3.2.8 crystal system, n - crystals are classified according to their crystallographic axes length and the
68 angles between them. There are six crystal systems: cubic, tetragonal, hexagonal, orthorhombic,
69 monoclinic, and triclinic. All crystal systems are anisotropic except for the cubic system, which is
70 isotropic.
71
72 3.2.9 epsilon (ϵ) - any vibration direction in the plane of the c axis for uniaxial crystals.
73
74 3.2.10 euhedral, adj - well-formed crystals bounded by plane faces.
75
76 3.2.11 extinction, n - condition when an anisotropic substance appears dark when observed between
77 crossed polarizers and occurs when the vibration directions of the specimen are parallel to the vibration
78 directions of the polarizer and analyzer.
79
80 3.2.12 extinction, oblique, n - a type of extinction in which the vibration directions are inclined at an
81 angle relative to a crystal face.
82
83 3.2.13 extinction, parallel, n - a type of extinction in which the vibration directions are parallel to the
84 crystal faces.
85
86 3.2.14 extinction, symmetrical, n - a type of extinction in which the vibration directions bisect a
87 prominent interfacial angle of the crystal
88
89 3.2.15 gamma (γ) - symbol representing the highest of the three principal refractive indices of a biaxial
90 crystal.
91
92 3.2.16 interference figure, n – pattern observed during conoscopic observation of an anisotropic material
93 which consists of a combination of extinction positions and interference colors corresponding to the full
94 cone of directions by which the sample is illuminated.
95
96 *Discussion*—Conoscopic observations are typically made by inserting a Bertrand lens into the body tube
97 or by removing an eyepiece and viewing down the body tube.
98

99 3.2.17 fusion methods, n - A process in which compounds are heated on a microscope slide and observed
100 via PLM during heating, melting, and, upon cooling, recrystallization of the melt.

101 *Discussion*—Fusion methods frequently employ a temperature controlled hot stage capable of at least
102 300^o Celsius placed on the stage of a polarizing light microscope.

103 3.2.18 omega (ω) - any vibration direction in the plane of the a axis for uniaxial crystals.

104 3.2.19 optic axial angle ($2V$), n - the acute angle between two optic axes of a biaxial crystal.

105
106 3.2.20 optic axis, n - a direction of isotropic light propagation in an anisotropic crystal. Uniaxial crystals
107 have one optic axis; biaxial crystals have two optic axes.

108
109 3.2.21 optic sign, n - determined by the relationship of the refractive indices of a material. For uniaxial
110 crystals, if $\epsilon > \omega$, the crystal is positive (+); if $\omega > \epsilon$, the crystal is negative (-). For biaxial crystals, if $\gamma - \beta$
111 $> \beta - \alpha$, the crystal is positive (+); if $\gamma - \beta < \beta - \alpha$, the crystal is negative (-).

112
113 3.2.22 polymorphism, n - crystallization into two or more chemically identical but crystallographically
114 distinct forms.

115
116 3.2.23 relief, n - contrast between a particle or crystal and its media due to the difference between their
117 refractive indices. The greater the numerical difference in refractive indices, the greater the relief.

118
119 3.2.24 subhedral, adj - crystals with imperfectly developed faces.

120
121 3.2.25 uniaxial, adj - an anisotropic crystal in the tetragonal or hexagonal system having one optic axis
122 (isotropic direction) and either two (tetragonal) or three (hexagonal) directions which are alike and
123 perpendicular to the direction of the optic axis.

124 125 **4. Summary of Practice**

126
127 4.1 Solid chemical components in samples are analyzed using a polarized light microscope (PLM). The
128 optical crystallographic properties such as refractive index, birefringence, and morphology are
129 determined. Determination of these properties, which are different for various explosive components and
130 other chemical compounds, can aid in the identification of explosives and explosive residues submitted to
131 the forensic laboratory.

132
133 4.2 Optical crystallographic properties of an unknown component can be determined by either mounting
134 the component in a refractive index liquid, recrystallizing the component from a small drop of solvent, or
135 recrystallizing the component from its melt, i.e., recrystallization occurring when a melted compound is
136 cooled. Recrystallized components can be isolated for further analysis using other analytical techniques
137 such as Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, or scanning electron
138 microscopy-energy dispersive x-ray spectroscopy (SEM-EDS).

139
140 4.3 Water-soluble components, such as inorganic salts used as oxidizers, are dissolved in a drop of water
141 and tested using reagents to form characteristic microcrystals. Microcrystal tests can aid in the
142 determination of anions and cations present in oxidizers and other components of explosives.

143
144 4.4 The optical crystallographic properties of an inorganic compound and microcrystal testing for its ions
145 are independent techniques that provide complementary information. A combination of tests that includes

146 the optical crystallographic characterization of a compound and microchemical tests to confirm the anion
147 and cation are used to identify oxidizers and other salts present in explosives.

148
149 4.5 A combination of tests that include optical crystallographic characterization or microchemical tests in
150 conjunction with other independent techniques are used to identify chemical components present in
151 explosives. Refer to ASTM E3253 and E3329 for the requirements for the identification of commonly
152 encountered explosives and chemical components present in explosive materials.

153 154 **5. Significance and Use**

155
156 5.1 This standard is designed to assist the analyst using polarized light microscopy for the examination of
157 test samples for the presence of explosives.

158
159 5.2 Intact explosives and post-blast residues that contain unconsumed explosive components or their solid
160 reaction products are suitable for this standard (E3253, E3329). Particles only a fraction of a millimeter
161 in size can be examined using this standard.

162
163 5.3 This standard is not suitable for the examination of smokeless powder or liquid explosives.

164
165 5.4 Some of the microscopical techniques described in this standard allow for the recovery of the
166 specimen for subsequent analysis, but others, such as microcrystal tests, are destructive.

167
168 5.5 Identifications of explosive-related components based on these properties shall be part of an analytical
169 scheme as described in ASTM E3253 and E3229.

170 171 **6. Apparatus**

172
173 6.1 Polarized light microscope - A compound microscope with a rotating stage, polarizing filters,
174 substage condenser, and compensator slot.

175
176 Note 1—Most commercially available polarized light microscopes should be adequate for the forensic analysis of
177 explosives and appropriate for use following this standard .

178
179 6.2 Objectives - Strain-free typically 10X, 20X, and 40X.

180 6.2.1 A 40X objective with a numerical aperture of 0.65 or higher is recommended to provide the widest
181 view of the back focal plane for conoscopic observations.

182 6.2.2 A 10X dispersion staining objective is required if performing dispersion staining.

183
184 6.3 Compensator - A full wave compensator for use with the microscope. Optional compensators include
185 quarter-wave plate and quartz wedge.

186
187 6.4 Hot stage - A temperature controlled hot stage for use with the microscope with a range of
188 approximately 30°C to 300°C.

189
190 6.5 Alcohol lamp

191 192 **7. Reagents and Materials**

193
194 7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. It is intended that all reagents
195 conform to the specifications of the Committee on Analytical Reagents of the American Chemical

- 196 Society. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high
197 purity to permit its use without lessening the accuracy of the determination.
198
199 7.2 10% platinum chloride (H_2PtCl_6) in water
200
201 7.3 zinc uranyl acetate
202
203 7.4 ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$)
204
205 7.5 squaric acid
206
207 7.6 potassium iodide (KI)
208
209 7.7 nitron sulfate
210
211 7.8 strychnine sulfate
212
213 7.9 0.3% methylene blue in deionized water (Methylene Blue Reagent)
214
215 7.10 20% potassium nitrate (KNO_3) in deionized water
216
217 7.11 saturated zinc sulfate (ZnSO_4) in deionized water
218
219 7.12 Toluene or methylene chloride
220
221 7.13 Sodium hydroxide
222
223 7.14 Deionized water
224
225 7.15 Reference materials, including reagent grade chemicals and explosive materials, can be obtained
226 from commercial and retail sources or directly from the distributor or manufacturer.

227 7.16 Refractive index liquids with known temperature coefficients (dn/dt) and tolerance at n_D
228
229 7.17 Microscope slides and coverslips
230
231 7.18 Glass rod with a tapering tip of approximately 1 mm or less in diameter
232
233 7.19 Wood toothpicks
234
235 7.20 Glass ring with an approximate diameter of 10-12 mm and 5-10 mm in height
236
237 7.21 Tungsten needles
238
239 7.22 589 nm light filter
240
241 **8. Initial Microscopical Examination**

242 8.1. Preliminary assessment of the samples using visual and stereo microscopical examinations are
243 conducted following E3253-21 and E3329-21. If preliminary examinations indicate that the sample is an

244 explosive mixture or a component thereof, a portion of the sample is examined by PLM in order to obtain
245 further information regarding the composition of the sample.

246 8.2 One to three particles or grains (approximately 1 mg or less) are mounted on a glass microscope slide
247 with a cover slip and dispersed in a refractive index oil. For post-blast evidence a similar quantity of
248 residue material is mounted in the same manner. Any refractive index oil in the 1.400 to 1.800 range
249 should be used, however, if preliminary examinations indicate a particular explosive material, e.g., black
250 powder, ammonium nitrate-fuel oil (ANFO) or Composition C-4, the examiner should select a refractive
251 index oil that will enable confirmation of characteristic optical crystallographic data based upon Tables 1
252 and 2.

253 8.3 The mounted sample is examined with a polarized light microscope at low magnification (e.g. 10X).
254 The magnification is increased as needed to better view particles for examination.

255 8.4 Charcoal, if present, will appear as irregularly shaped, black and opaque particles, and as brown flakes
256 often with cellular features such as pits or cell walls (12).

257 8.5 Most common explosives and oxidizers are colorless when viewed microscopically with transmitted
258 plane polarized light. Crossed polarizers are used to determine if the crystals are isotropic or anisotropic.
259 Interference colors are assessed to determine if the birefringence is low (less than ~ 0.01), moderate
260 ($\sim 0.01-0.10$) or high (above ~ 0.10).

261 8.6 The relative refractive indices of the crystals present in the specimen are determined using the Becke
262 line method or dispersion staining.

263 8.7 The morphology, relative refractive indices, and interference colors can indicate the presence of an
264 explosive ingredient or chemical component. See Tables 1 and 2.

265 8.7.1 If explosive related components are indicated, additional microscopical analysis is performed to
266 further characterize the components via optical crystallography (Sections 9 & 10).

267 8.7.2 If inorganic salts are indicated, additional microscopical analysis may be performed by microcrystal
268 tests (Section 11).

269 8.7.3 If no explosive related components are indicated, further analysis using microscopy or other
270 instrumental analyses could be performed to characterize or identify the substance in the case sample.
271 Such examinations are beyond the scope of this document.

272 **9. Optical Crystallography by Solvent Recrystallization**

273 9.1 Solvent recrystallization allows for a more detailed optical crystallographic characterization of a
274 compound as it recrystallizes from a small drop of suitable solvent.

275 9.2 Water recrystallization for inorganic oxidizers and salts

276 9.2.1 A small drop (5-7 mm in diameter) of distilled or deionized water is placed on a microscope slide.
277 1-3 particles or grains (approximately 1 mg or less) from the sample with similar appearance are added to
278 the drop. If the specimen does not readily dissolve, it should be crushed and dispersed with the tip of a
279 glass rod to aid dissolution.

280 9.2.1.1 Alternatively, if a large amount of insoluble components are present, a portion of the specimen
281 can be mixed with water and filtered to remove water insoluble components such as carbon, aluminum,
282 and sulfur. A small drop of the filtered water extract is then placed on a microscope slide.

283 9.2.2 The test drop is examined using the PLM and a low magnification objective, e.g., 10X. Crystals
284 typically form at the edge of the drop first. Distorted or subhedral crystals, if formed, are pushed back
285 into the center of the drop using a device such as a glass rod, toothpick or metal probe to produce
286 characteristic euhedral crystals. Multiple water-soluble components can be present in the sample and,
287 depending on their concentration and solubility, could recrystallize at different times as the drop
288 evaporates.

289 9.2.3 Crystals formed as the drop evaporates are examined using plane-polarized and crossed-polarized
290 light. Observe their crystal habit, relief, and whether they are isotropic or anisotropic.

291 9.2.4 For anisotropic euhedral crystals, extinction is determined to be parallel, symmetrical, or oblique.

292 9.2.5 Interference colors are assessed to determine if the birefringence is low (less than ~ 0.01), moderate
293 ($\sim 0.01-0.10$), or high (above ~ 0.10).

294 9.2.6 Observable interference figures are used to determine if the crystal is uniaxial or biaxial and the
295 optic sign. Interference figures can also be used to relate morphology of the crystal to its optical
296 orientation, and, if the crystal is biaxial, estimate the optic axial angle ($2V$).

297 9.2.7 The optical crystallographic properties of the crystals are documented by description, sketch, or
298 photomicrographs.

299 9.2.8 The optical crystallographic properties of the crystals formed are compared to recrystallized known
300 reference materials or published references (6, 8). A tentative identification is made if the optical
301 crystallographic properties of the specimen are the same as a given compound. Table 1 lists optical
302 crystallographic properties for twelve common oxidizers.

303 9.2.9 If two or more oxidizers recrystallize from the drop, their tentative identification is compared to the
304 optical properties determined from examinations of the intact sample (Section 8 and 10) to assess if a
305 metathetical reaction has occurred. If a metathetical reaction occurred, the optical properties of the intact
306 crystals and those obtained from recrystallization will be different.

307 9.2.10 Depending on the analytical scheme chosen, dried crystals can be 1) mounted in refractive index
308 oils for refractive index determination (Section 10), 2) analyzed via microcrystal tests (Section 11), or 3)
309 analyzed by other instrumental techniques (E3253, E3329) If the crystals cannot be further analyzed on
310 the glass slide, a tungsten needle is used to transfer the crystals from the glass slide to another substrate
311 such as a potassium bromide pellet for additional analysis.

312 9.3 Organic solvent recrystallization for sulfur

313 9.3.1 A small drop (5-7 mm in diameter) of a suitable solvent, such as toluene or methylene chloride, is
314 placed on a microscope slide. One to three particles or grains (approximately 1 mg or less) of sample are
315 added to the solvent drop. Sulfur, if present, will recrystallize as highly refractive, birefringent
316 dipyramids (diamond-shaped) or form liquid drops that crystallize when touched with a tungsten needle.

317 9.3.2 The optical crystallographic properties of the crystals are documented by description, sketch, or
318 photomicrograph.

319 9.3.3 The optical crystallographic properties of the crystals formed are compared to recrystallized known
320 reference materials or published references (5, 6). A tentative identification is made if the optical
321 crystallographic properties of the specimen are the same as a given compound. Table 1 lists optical
322 crystallographic properties for sulfur.

323 9.3.4 Depending on the analytical scheme chosen, dried crystals can be further analyzed by other
 324 analytical techniques (E3253, E3329). If the crystals cannot be further analyzed while on the glass slide,
 325 a tungsten needle is used to transfer crystals from the glass slide to another substrate such as carbon tape
 326 for additional analysis.

327 10. Optical Crystallography Using Refractive Index Liquids

328 10.1 Organic and inorganic compounds present in explosives are characterized by their optical
 329 crystallographic properties by mounting intact material in a liquid of known refractive index.

330 10.2 One to three particles or grains (approximately 1 mg or less) of the sample, or dried crystals obtained
 331 during recrystallization, as described in Section 9, are mounted in a refractive index oil in the 1.400 to
 332 1.800 range. If previous examinations indicate a particular compound, e.g., potassium nitrate or RDX, the
 333 examiner should select a refractive index oil that will provide characteristic optical crystallographic data
 334 based on Tables 1 and 2.

335 10.3 The crystals are examined using PLM to determine the optical crystallographic properties such as
 336 refractive indices, birefringence, extinction type (parallel, symmetrical, or oblique), optic sign, and optic
 337 axial angle (2V).

338 10.4 Refractive index can be determined using the Becke line method or dispersion staining. The
 339 specimen can be mounted in additional refractive index liquids to confirm one or more refractive indices
 340 of the indicated compound.

341 10.5 The optical crystallographic properties of the specimen are compared to known reference materials
 342 or published references (e.g., 5, 6, 8, 12-16, 22). A tentative identification is made if the optical
 343 crystallographic properties of the specimen are the same as a given compound. Table 1 lists optical
 344 crystallographic properties for three fuels (sulfur, ascorbic acid and sucrose) and twelve common
 345 oxidizers. Table 2 lists optical crystallographic properties for four organic high explosives.

346

347 **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°(+)	dipyramids	High relief in solvent. Larger crystals appear yellowish in color.
Ascorbic Acid (22)	monoclinic	1.474 1.595 1.746 2V = 88°(+)		High order interference colors
Sucrose (12, 22)	monoclinic	1.540 1.567 1.572 2V = 48°(-)		
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 ₄ ClO 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 ₂ O	Sodium chlorate recrystallizes as the hydrate. Optical data is for the anhydrate.
Sodium perchlorate dihydrate (NaClO ₄ .2H ₂ O) (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 ₄ NO 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.

348

349

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° (+)	dipyramids & pinacoids, rare acicular	Typically exhibits high order interference colors. Oblique extinction
TNT (13)	orthorhombic	1.543 1.674 1.717 2V = 60° (-)		MP = 81C
PETN (13)	tetragonal	$\omega = 1.556$ $\varepsilon = 1.551$		MP = 141.5-142C

350

351 11. Microcrystal tests

352 11.1 Microcrystal tests are chemical precipitation reactions in which the target ion forms an insoluble
353 reaction product with the reagent. The crystals formed are examined using a polarized light microscope.
354 Microcrystal tests are used in conjunction with other independent techniques to identify explosive related
355 compounds.

356 11.2 Microcrystal tests are used to test for anions and cations of oxidizers and other substances such as
357 aluminum that are present in explosives. Many microcrystal tests are available, those commonly used in
358 explosives analysis are listed in Table 3.

359 11.3 The presence of additional water-soluble compounds can interfere with, or affect the formation of,
360 microcrystals of the target anion or cation. If this occurs, further isolate the target compound and repeat
361 the microcrystal test or use a different analytical technique.

362 11.4 Prior to casework, the reagents used for the microcrystal tests are tested for reliability using the
363 appropriate positive and negative controls. The crystals obtained with the positive control are compared
364 to the published references (1, 4, 7, 17, 19, 20, 21).

365 11.5 There are four routine methods for conducting microcrystal tests (4). Test drops and reagent drops
366 used for microcrystal tests should be approximately 5-7 mm in diameter or smaller. Most reactions take
367 place within one minute. Test drops with lower concentrations may take longer to occur.

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

369 11.5.1 Method 1

370 11.5.1.1 A drop of deionized water is placed on a clean microscope slide and one or more particles of the
371 sample is added with stirring, crushing and dispersing as needed to dissolve the water-soluble
372 components. The test drop should be kept small. Alternatively, 1-5 grains or particles of the sample are
373 placed in a spot well plate or test tube and 3-4 drops of deionized water are added. The specimen is
374 stirred with a glass rod to disperse and dissolve any water-soluble components. A test drop of the
375 aqueous solution is placed on a clean microscope slide.

376 11.5.1.2 Place a drop of reagent near, but not touching, the test drop. Depending on the microcrystal test
377 being conducted some reagent solutions are pre-made and a drop added to the slide. Other reagent drops
378 are created on the slide by placing a drop of water near the test drop and adding 1-5 particles of the
379 reagent to the water droplet.

380

381 11.5.1.3 The test drop and reagent drop are drawn together using a clear glass rod or toothpick. The
382 preparation is immediately examined microscopically using a 10X objective. Higher magnification can
383 be used but care must be taken to ensure the objective does not contact the test drop.

384 11.5.1.4 The preparation is examined for the presence of characteristic microcrystals or the lack of a
385 reaction.

386 11.5.1.5 The formation of microcrystals corresponding to those obtained with the positive control
387 indicates the presence of that anion or cation.

388

389 11.5.2 Method 2

390 11.5.2.1 Follow 11.5.1 except the reagent drop is added to the middle of the test drop.

391 11.5.2.2 The preparation is examined microscopically as described in Method 1 for the presence of
392 characteristic microcrystals or the lack of a reaction.

393 11.5.2.3 The formation of microcrystals corresponding to those obtained with the positive control
394 indicates the presence of that anion or cation.

395

396 11.5.3 Method 3

397 11.5.3.1 Follow 11.5.1 except add reagent particles into the middle of the test drop.

398 11.5.3.2 The preparation is examined microscopically as described in Method 1 for the presence of
399 characteristic microcrystals or the lack of a reaction.

400 11.5.3.3 The formation of microcrystals corresponding to those obtained with the positive control
401 indicates the presence of that anion or cation.

402

403 11.5.4 Method 4

404 11.5.4.1. A small drop of deionized water is placed on a clean microscope slide. One or more particles of
405 the sample is added to the drop with stirring, crushing, and dispersing as needed to dissolve the water-
406 soluble components. The test drop should be kept small.

407 11.5.4.2 A glass ring with an internal diameter of about 10-12 mm is placed around the test drop so the
408 drop is in the center of the ring.

409 11.5.4.3 A small drop of the prepared reagent is placed on a coverslip.

410 11.5.4.4 A small drop of the volatilizing solution (e.g., 10% sodium hydroxide) is added to the test drop
411 inside the center of the ring.

412 11.5.4.5 The coverslip is immediately placed onto the glass ring such that the reagent drop is suspended
413 in the center of the ring above the sample.

414 11.5.4.6 The slide preparation is placed on the microscope stage ensuring sufficient working distance
415 between the top of the glass ring and the objective.

416 11.5.4.7 The reagent drop is examined for the presence of microcrystals or the lack of reaction.

417 11.5.4.8 The formation of microcrystals corresponding to those obtained with the positive control
418 indicates the presence of that anion or cation.

419

420 11.6 Table 3 lists twelve tests for anions and cations common to low explosives along with the reagent,
421 literature reference, and method as described in 11.5. Microcrystal tests for additional ions can be found
422 in Chamot & Mason (4), Benedetti-Pichler (21), Wills (19), and Hollifield (20).

423

424

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

425

426 12. Fusion Methods

427 12.1 Fusion methods (10) are techniques that allow for the characterization of crystals by determining the
428 melting point (MP) and observing optical crystallographic properties as the sample recrystallizes from the
429 melt. This method is most useful for organic high explosives (11, 14, 15, 16) but can also be applied to
430 some inorganic explosives (9). Crystalline compounds examined using fusion methods must first be
431 isolated from mixtures by physical or chemical separation.

432 12.2 Examination Using Temperature Controlled Hot Stage

433 12.2.1 The isolated crystalline substance is placed on a microscope slide with a cover slip, or on a cover
434 slip (according to the hot stage manufacturer's instructions).

435 12.2.2 The preparation is placed in the hot stage at a temperature of approximately 50C. If a particular
436 explosive compound has been indicated from prior analysis, a temperature approximately 10-20C lower
437 than the expected melting point can be used.

438 12.2.3 The specimen is brought into the field of view. The temperature of the hot stage is raised while the
439 specimen is viewed using the polarized light microscope. The melting point is recorded. The temperature
440 is slowly lowered while observing the recrystallization of the sample from the melt.

441 12.2.4 The optical crystallographic properties such as morphology, crystal front shape, polymorphism,
442 interference colors, anomalous interference colors, extinction characteristics, interference figure, and
443 optic sign are observed for each solid state as appropriate.

444 12.3 Alternatively, the sample is heated on a microscope slide using an alcohol lamp (9). This method is
445 especially useful for substances with melting points above 300C, including most inorganic oxidizers. An
446 accurate melting point is not determined, but the sample is observed through the microscope as the slide
447 cools to obtain various optical crystallographic properties, as described in 12.2.4.

448 12.4 Compare the optical crystallographic properties of the specimen to published reference
449 descriptions (9-11, 14-16) or a known reference material.

450 12.5 Mixed fusions

451 12.5.1 If an explosive compound is indicated using optical crystallography, microcrystal tests, or other
452 techniques, a mixed fusion using a known reference material of the suspected compound can confirm the
453 identity of the unknown (9, 10).

454

455 12.5.2 A small amount of isolated crystalline substance from the sample is placed on a slide, under a
456 coverslip, but near the edge of the coverslip. The sample is melted over an alcohol lamp. The melted
457 sample should fill approximately half of the area under the coverslip.

458 12.5.3 Once the unknown sample has cooled and recrystallized, the known reference material is placed
459 near the opposite edge of the coverslip and melted over an alcohol lamp such that the melted standard
460 flows under the coverslip and contacts the previously cooled unknown specimen. The portion of the
461 unknown specimen that contacts the reference material will also melt during this process.

462 12.5.4 Using the PLM, the zone of mixing is viewed as the preparation cools and recrystallizes. If the
463 unknown compound is the same as the reference material, the crystals of the unknown will continue to
464 grow through the zone of mixing with no change in crystal growth rate or in appearance. If the unknown
465 compound and reference material are different, a change in the crystal growth rate occurs and the crystals
466 in the zone of mixing usually appear different.

467 12.5.5 Once cooled, the mixed fusion preparation can be inserted into the hot stage at a temperature
468 approximately 10 degrees lower than the melting point of the reference material. While observing the
469 zone of mixing, slowly raise the temperature (1-3 degrees/minute) of the hot stage to the melting point of
470 the reference material. If the two compounds are the same, the melting point will be the same across the
471 zone of mixing.

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

473 **13. Documentation**

474 13.1 Retain all notes and supporting analytical data used for the identification or tentative identification of
475 explosive related compounds in accordance with E620, E860 and E3255. Examples of such data include
476 photomicrographs and detailed descriptions of microscopical observations.

477 13.2 Record case notes in sufficient detail such that an independent analyst could understand and evaluate
478 the work performed, independently interpret the data, and form an opinion.

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