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ISO/ASTM 51538:2009(E)



Standard Practice for Use of the Ethanol-Chlorobenzene Dosimetry System¹

This standard is issued under the fixed designation ISO/ASTM 51538; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision.

1. Scope

- 1.1 This practice covers the procedure for preparation, handling, testing, and use of procedure for using the ethanol-chlorobenzene (ECB) dosimetry system to determinemeasure absorbed dose (in terms of absorbed dose to water) in materials irradiated by photons (gamma radiation or X-radiation/bremsstrahlung) or high energy electrons. to water when exposed to ionizing radiation. The system consists of a dosimeter and appropriate analytical instrumentation. For simplicity, the system will be referred to as the ECB system. It The ECB dosimeter is classified as a reference-standard dosimetry system and is also type I dosimeter on the basis of the effect of influence quantities. The ECB dosimetry system may be used as a routine reference standard dosimetry system (see ISO/ASTM Guideor as a 51261), routine dosimetry system.
- 1.2 This document is one of a set of standards that provides recommendations for properly implementing dosimetry in radiation processing, and describes a means of achieving compliance with the requirements of ISO/ASTM Practice 52628 for the ECB system. It is intended to be read in conjunction with ISO/ASTM Practice 52628.
- 1.3 This practice describes the mercurimetric titration analysis as a standard readout procedure for the ECB dosimeter when used as a reference standard dosimetry system. Other readout methods (spectrophotometric, oscillometric) that are applicable when the ECB system is used as a routine dosimetry system are described in Annex A1 and Annex A2 and Annex A1.
 - 1.4 This practice applies only to gamma radiation, X-radiation/bremsstrahlung, and high energy electrons.
 - 1.5 This practice applies provided the following conditions are satisfied:
- 1.5.1 The absorbed dose range is between 10 Gy and 2 MGy for gamma radiation and between 10 Gy and 200 kGy for high current electron accelerators (1,_2). (Warning—the boiling point of ethanol chlorobenzene solutions is approximately 80°C. 80°C. Ampoules may explode if the temperature during irradiation exceeds the boiling point. This boiling point may be exceeded if an absorbed dose greater than 200 kGy is given in a short period of time.)
 - 1.5.2 The absorbed-dose rate is less than 10^6 Gy s⁻¹(2).
- 1.5.3 For radionuclide gamma-ray sources, the initial photon energy is greater than 0.6 MeV. For bremsstrahlung photons, the energy of the electrons used to produce the bremsstrahlung photons is equal to or greater than 2 MeV. For electron beams, the initial electron energy is equal to or greater than 48 MeV (3)). (see ICRU Reports 34 and 35).
- Note 1—The same response relative to ⁶⁰Co gamma radiation was obtained in high-power bremsstrahlung irradiation produced by a 5 MeV electron accelerator (4).
- Note 2—The same response relative to ⁶⁰Co gamma radiation was obtained in high-power bremsstrahlung irradiation produced by a 5 MeV electron accelerator (4). The lower limits of energy givenlimits are appropriate for a cylindrical dosimeter ampoule of 12-mm diameter. Corrections for dose gradients across an ampoule of that diameter or less are not required, the ampoule may be required for electron beams. The ECB system may be used at energies of incident electrons lower than 4 MeV lower energies by employing thinner (in the beam direction) dosimeters. dosimeters (see ICRU Report 35). The ECB system may also be used at X-ray energies as low as 120 kVp (5). However, in this range of photon energies the effect caused by the ampoule wall is considerable.
- Note 3—The effects of size and shape of the dosimeter on the response of the dosimeter can adequately be taken into account by performing the appropriate calculations using cavity theory (6).
 - 1.5.4 The irradiation temperature of the dosimeter is within the range from -40°C to 80°C.-30 °C to 80 °C.
- Note 4—The temperature dependence of dosimeter response is known only in this range (see 4.35.2). For use outside this range, the dosimetry system should be calibrated for the required range of irradiation temperatures.

¹ This practice is under the jurisdiction of ASTM Committee E61 on Radiation Processing and is the direct responsibility of Subcommittee E61.02 on Dosimetry Systems, and is also under the jurisdiction of ISO/TC 85/WG 3.

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² The boldface numbers in parentheses refer to the bibliography at the end of this practice.

ISO/ASTM 51538:2017(E)





- 1.4 The effects of size and shape of the dosimeter on the response of the dosimeter can adequately be taken into account by performing the appropriate calculations using cavity theory (6).
- 1.6 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. Specific warnings are given in 1.5.1, 8.29.2 and 9.210.2.
- 1.7 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced documents

2.1 ASTM Standards:³

C912 Practice for Designing a Process for Cleaning Technical Glasses

D1193 Specification for Reagent Water

E170 Terminology Relating to Radiation Measurements and Dosimetry

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

E666 Practice for Calculating Absorbed Dose From Gamma or X Radiation

E668 Practice for Application of Thermoluminescence-Dosimetry (TLD) Systems for Determining Absorbed Dose in Radiation-Hardness Testing of Electronic Devices

E925 Practice for Monitoring the Calibration of Ultraviolet-Visible Spectrophotometers whose Spectral Bandwidth does not Exceed 2 nm

E958 Practice for Estimation of the Spectral Bandwidth of Ultraviolet-Visible Spectrophotometers

2.2 ISO/ASTM Standards:³

51261 Guide Practice for Selection and Calibration of Routine Dosimetry Systems for Radiation Processing

51707 Guide for Estimation of Measurement Uncertainty in Dosimetry for Radiation Processing

5140052628 Practice for Characterization and Performance of a High-Dose Gamma-Radiation Dosimetry Calibration Laboratory Dosimetry in Radiation Processing

5170752701 Guide for Estimating Uncertainties in Dosimetry for Performance Characterization of Dosimeters and Dosimetry Systems for Use in Radiation Processing

2.3 ISO Standards:⁴

12749-4 Nuclear energy – Vocabulary – Part 4: Dosimetry for radiation processing

2.4 ISO/IEC Standards:⁴

17025 General Requirements for the Competence of Testing and Calibration Laboratories

2.5 Joint Committee for Guides in Metrology (JCGM) Reports:

JCGM 100:2008, GUM 1995, with minor correctons Evaluation of measurement data – Guide to the Expression of Uncertainty in Measurement⁵

<u>JCGM 200:2012, (JCGM 200:2008 with minor revisions) VIM, International Vocabulary of Metrology – Basis and General Concepts and Associated Terms⁶</u>

2.6 International Commission on Radiation Units and Measurements (ICRU) Reports:⁷

ICRU Report 14 Radiation Dosimetry: X-Rays and Gamma Rays with Maximum Photon Energies Between 0.6 and 60 MeV

ICRU Report 17 Radiation Dosimetry: X-Rays Generated at Potentials of 5 to 150 kV

ICRU Report 34 The Dosimetry of Pulsed Radiation

ICRU Report 35 Radiation Dosimetry: Electrons with Initial Energies Between 1 and 50 MeV

ICRU Report 3780 Stopping Powers for Electrons and Positrons Dosimetry Systems for Use in Radiation Processing

ICRU Report 6085a Fundamental Quantities and Units for Ionizing Radiation

3. Terminology

3.1 Definitions:

3.1.1 approved laboratory—laboratory that is a recognized national metrology institute, or has been formally accredited to ISO/IEC 17025, or has a quality system consistent with the requirements of ISO/IEC 17025.

³ For referenced ASTM and ISO/ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

⁴ Available from International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

⁵Document produced by Working Group I of the Joint Committee for Guides in Metrology (JCGM WG1). Available free of charge at the BIPM website (http://www.bipm.org).

⁶Document produced by Working Group 2 of the Joint Committee for Guides in Metrology (JCGM WG2). Available free of charge at the BIPM website (http://www.bipm.org).

Available from the Commission on Radiation Units and Measurements, 7910 Woodmont Ave., Suite 800, Bethesda, MD 20814, USA.



3.1.1.1 Discussion—

A recognized national metrology institute or other calibration laboratory accredited to ISO/IEC 17025 should be used in order to ensure traceability to a national or international standard. A calibration certificate provided by a laboratory not having formal recognition or accreditation will not necessarily be proof of traceability to a national or international standard.

3.1.2 *calibration*—set of operations <u>that establish</u>, under specified conditions, which establishes the relationship between values indicated by a measuring instrument or measuring system, and the corresponding values realised by standards traceable to a nationally or internationally recognized laboratory. <u>or values represented by a material measure or a reference material</u>, and the corresponding values realised by standards.

3.1.2.1 Discussion—

Calibration conditions include environmental and irradiation conditions present during irradiation, storage and measurement of the dosimeters that are used for the generation of a calibration curve. To achieve stable environmental conditions, it may be necessary to condition the dosimeters before performing the calibration procedure.

3.1.3 calibration curve—graphical representation of the dosimetry system's response function.expression of the relation between indication and corresponding measured quantity value.

3.1.3.1 Discussion—

In radiation processing standards, the term "dosimeter response" is generally used for "indication".

- 3.1.4 *dosimetry system*—system used for determining absorbed dose, consisting of dosimeters, measurement instruments and their associated reference standards, and procedures for the system's use.
- 3.1.5 *ethanol-chlorobenzene dosimeter*—partly deoxygenated solution of chlorobenzene (CB) in 96 volume % ethanol in an appropriate container, such as a flame-sealed glass ampoule, used to indicate absorbed dose by measurement of the amount of HCl formed under irradiation.
- 3.1.6 measurement management system—set of interrelated or interacting elements necessary to achieve metrological confirmation and continual control of measurement processes.
- 3.1.7 measurement quality assurance plan—metrological traceability—documented program for the measurement process that ensures that the expanded uncertainty consistently meets the requirements of the specific application. This plan requires traceability to nationally or internationally recognized standards, property of a measurement whereby the result can be related to a reference through a documented unbroken chain of comparisons, each contributing to the measurement uncertainty.
- 3.1.8 molar linear absorption coefficient ε_m —constant relating the spectrophotometric absorbance, A_{λ} , of an optically absorbing molecular species at a given wavelength, λ , per unit pathlength, d, to the molar concentration, c, of that species in solution:

$$\varepsilon_m = \frac{A_{\lambda}}{d \times c} \tag{1}$$

$$\epsilon_m = \frac{A_\lambda}{d \times c} \tag{1}$$

(SI unit: m² mol⁻¹)

3.1.8.1 Discussion—

It is sometimes expressed in units of L mol⁻¹ cm⁻¹.

3.1.9 radiation chemical yield G(x)—quotient of $n(x)\underline{n(x)}$ by $\bar{\varepsilon}$ where $n(x)\underline{n(x)}$ is the mean amount of a specified entity, x, produced, destroyed, or changed by the mean energy, $\bar{\varepsilon}_m$ imparted to the matter.

$$G(x) = n(x)/\bar{\varepsilon} \tag{2}$$

(SI unit: $mol J^{-1}$)

- 3.1.10 reference-standard dosimeter—reference standard dosimetry system—dosimeter of high metrological quality used as a standard to provide measurements traceable to measurements made using primary-standard dosimeters:dosimetry system, generally having the highest metrological quality available at a given location, from which measurements made there are derived.
- 3.1.9 response function—mathematical representation of the relationship between dosimeter response and absorbed dose, for a given dosimetry system.

ISO/ASTM 51538:2017(E)





- 3.1.11 routine <u>dosimeter—dosimetry system—dosimeter dosimetry system</u> calibrated against a <u>primary-, reference-, or transfer-standard dosimeter reference standard dosimetry system</u> and used for routine absorbed-dose <u>measurements.measurements</u>, including dose mapping and process monitoring.
- 3.1.12 *traceability*—<u>type 1 dosimeter</u>—<u>property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties. dosimeter of high metrological quality, the response of which is affected by individual influence quantities in a well-defined way that can be expressed in terms of independent correction factors.</u>
 - 3.2 Definitions of Terms Specific to This Standard:
 - 3.2.1 conductometry—analytical method based on the measurement of conductivity of solutions.

3.2.1.1 Discussion—

The conductivity of a solution depends on the concentration of free ions in the solution.

- 3.2.2 oscillometry—electroanalytical method of conductivity measurements, when high-frequency (1 to 600 MHz) alternating current is applied to measure or follow changes in the composition of chemical systems.
- 3.3 Definitions of other terms used in this standard that pertain to radiation measurement and dosimetry may be found in <u>ISO</u> <u>12749-4</u>, ASTM Terminology <u>Standard E170</u>. Definitions in ASTM E170 are compatible with ICRU 60; that document, ICRU 85a and VIM; these definitions, therefore, may be used as an alternative <u>reference</u>.references.

4. Significance and use

- 4.1 The ECB dosimetry system provides a reliable means of measuring absorbed dose in materials. to water. It is based on a process of radiolytic formation of hydrochloric acid (HCl) in aqueous ethanolic solutions of chlorobenzene by ionizing radiation ((7, 8).), ICRU 80).
- 4.2 The dosimeters are partly deoxygenated solutions of chlorobenzene (CB) in 96 volume % ethanol in an appropriate container, such as a flame-sealed glass ampoule. Radiation chemical yields (G) for the formation of HCl in typical ECB solution formulations are given in Table 1.
- 4.3 The dosimeters are partly deoxygenated solutions of chlorobenzene (CB) in 96 volume % ethanol in an appropriate container, such as a flame-sealed glass ampoule. The irradiated solutions indicate absorbed dose by the amount of HCl formed. A number of analytical methods are available for measuring the amount of HCl in ethanol (910).

4.3 Effect of Irradiation Temperature:

4.3.1 The temperature dependence of dosimeter response is a complex function of dose and temperature for each concentration of chlorobenzene (that is, for each formulation). The analysis of the published data (10) shows that the temperature dependence between 20°C and 80°C at any chlorobenzene concentration can be described by a simple exponential expression:

 $G_t = G_0 \exp[k(t - 20)] \tag{3}$

where:

TABLE 1 Typical Radiation chemical yields (G) for the formation of HCl in typical ECB solution formulations

Concentration of CB, vol %	Density at 20°C 20 °C kg · m ⁻³	Datie of	Radiation Chemical Yields at $\frac{20^{\circ}\text{C}20~^{\circ}\text{C}^{B}}{\underline{\cdot}\text{J}^{-1}}$		
		Ratio of Coefficients ^A	⁶⁰ Co Gamma	4 to 10 MeV Electrons (3)	
		F	Rays Radiation	` ,	
			(11 <u>9</u>)		
4	819	0.989	0.42 ^C	_	
10	839	0.995	0.52		
20	869	1.006	0.59		
24	880	1.011	0.60	0.57 ^D	
40	925	1.027	0.63		

A The ratio of the <u>photon</u> mass energy-absorption coefficients for water and the dosimeter solution at ⁶⁰Co gamma ray energy:

$$f = \frac{(\mu_{er}/\rho)_w}{(\mu_{er}/\rho)_D}$$

^B Radiation chemical yieldsyield of HCl in the dose range from 100 Gy to 100 kGy.

^C Upper dose range 20 kGy.

 $^{^{}D}$ Lower dose range 1 kGy. This formulation also contained 0.04 % acetone and 0.04 % benzene.



- = the radiation chemical yield in μ mol J⁻¹ at a given temperature t in ${}^{\circ}$ C,
- G_0 = the radiation chemical yield in μ mol J⁻¹ at 20°C (G_0 for different ECB solutions are given in Table 1), and
- = the temperature coefficient in (°C)⁻¹ applicable at a given dose.
 - 4.3.2 The values of k are given in Table 2.
- 4.3.3 Between −30°C and 50°C the temperature coefficient 0.015 kGy/°C applies at 30 kGy dose (12). Information on the temperature dependence of dosimeter response during irradiation between 20 and 80°C is found in Ref (10), and between -40 and 20°C in Ref (13).
- 4.4 The concentration of chlorobenzene in the solution can be varied so as to simulate a number of materials in terms of the photon mass energy-absorption coefficients (μ_{en}/ρ) for X- and gamma radiation, and electron mass collision stopping powers (S/ ρ), over a broad energy range from 10^{-2} to 100 MeV (14-11-1714).
- 4.5 The absorbed dose that is determined is the dose absorbed in the water. Absorbed dose in other materials irradiated under equivalent conditions may be calculated. Procedures for making such calculations are given in ASTM Practices E666 and E668 and ISO/ASTM Guide 51261.

Note 3—For a comprehensive discussion of various dosimetry methods applicable to the radiation types and energies discussed in this practice, see ICRU Reports 14, 17, 34, 35, and 37.

4.5 The ECB dosimetry system may be used with other radiation types, such as neutrons (1815), and protons (1916). Meaningful dosimetry of any radiation types and energies novel to the system's use requires that the respective radiation chemical responses applicable under the circumstances be established in advance.

5. Effect of Influence Quantities

- 5.1 Guidance on the determination of the performance characteristics of dosimeters and dosimetry systems can be found in ISO/ASTM Guide 52701. The relevant influence quantities that need to be considered when using the ECB dosimetry system are given below.
- 5.2 The irradiation temperature dependence of dosimeter response is a complex function of dose and temperature for each concentration of chlorobenzene (that is, for each formulation). This dependence arises directly from the temperature dependence of radiation chemical yield, G. The analysis of the published data (17) shows that the irradiation temperature dependence of G between 20 °C and 80 °C at any chlorobenzene concentration can be described by a simple exponential expression:

 $G = G_0 \exp[k(t-20)]$ (3)

where:

- $G_t = \frac{\text{radiation chemical yield in } \mu \text{mol } J^{-1} \text{ at a given temperature } t \text{ in } ^{\circ}\text{C},$ $G_0 = \frac{\text{radiation chemical yield in } \mu \text{mol } J^{-1} \text{ at 20 } ^{\circ}\text{C} \text{ (}G_0 \text{ for different ECB solutions are given in Table 1), and}$ $k = \frac{k}{L} = \frac{\text{radiation chemical yield in } \mu \text{mol } J^{-1} \text{ at 20 } ^{\circ}\text{C} \text{ (}G_0 \text{ for different ECB solutions are given in Table 1), and}$ $k = \frac{L}{L} =$
 - 5.2.1 The values of k are given in Table 2.

6. Interferences

- 6.1 The ECB dosimetric solution response is not particularly sensitive to impurities which occur in commercially available components, chlorobenzene and ethanol of the analytical reagent (AR) grade purity or equivalent (pro analysi, p.a., and puriss). For high-accuracy results, organic materials of technical grade purity (or purum) can be purified by distillation.
- 6.2 Care should be exercised in filling ampoules to avoid depositing solution in the ampoule neck. Subsequent heating during sealing of the ampoule may cause an undesirable chemical change in the dosimetric solution remaining inside the ampoule's ampoule neck. Test tubes with ground-glass stoppers are therefore preferred to sealed ampoules for measuring doses below 100 Gy. For the same reason, care should be given to avoid heating the body of the ampoule during sealing.
- 6.3 The dosimetric solution is somewhat sensitive to ultraviolet light and should be kept in the dark for long-term storage. No special precautions are required during routine handling under normal laboratory lighting conditions, but strong ultraviolet (UV) sources such as sunlight should be avoided (2018).

TABLE 2 Temperature coefficients k (°C)⁻¹ for typical ECB solution formulations as derived from Ref (17)

Concentration of CB, Vol %	2.5 kGy	5 kGy	10 kGy	15 kGy	20 kGy	25 kGy
Concentration of CB, vol %	2.5 kGy	5 kGy	10 kGy	15 kGy	20 kGy	25 kGy
4	-0.0002	-0.0004	-0.0007	-0.0011	-0.0015	-0.0019
10	0.0018	0.0014	0.0009	0.0002	0.0	0.0
20, 25, 40	0.0037	0.0031	0.0020	0.0013	0.0008	0.0

ISO/ASTM 51538:2017(E)





Note 1—For For intermediate doses interpolation should be made.

7. Apparatus

- 7.1 This practice describes mercurimetric titration of radiolytically formed Cl⁻ ions as a standard readout procedure for the ECB system when used as a reference-standard dosimetry system.
- 7.2 For the analysis of the dosimetric solution, use a precision burette capable of measuring volumes with 0.01 mL resolution. If necessary, check the original calibration of volumetric glassware and, if necessary, recalibrate to attain 0.1 % relative uncertainty. Control the temperature of all solutions during handling at $20 \pm 2^{\circ}\text{C}$. 2 °C to ensure correct measurement of volumes.
- 7.3 Use borosilicate glass or equivalent chemically resistant glass to store the reagents and the prepared dosimetric solution, and to perform the titration. Clean all apparatus thoroughly before use (see ASTM Practice C912).
- 7.4 Use a sealed glass ampoule or other appropriate glass container to hold the dosimetric solution during irradiation. For photons, surround the container with material of thickness sufficient to produce approximate electron equilibrium conditions during calibration irradiations. For measurement of absorbed dose in water, use materials that have radiation-absorption properties essentially equivalent to water, for example, polystyrene and polyethylene. The appropriate thickness of such material depends on the energy of the photon (see ASTM Practices E666 and E668).

Note 5—The dosimetric ampoule commonly used has a capacity of about 5 mL. Quick-break, glass ampoules or "Type 1 glass" colorbreak ampoules or equivalent containers, may be used. Commercially available pharmaceutical ampoules have been found to give reproducible results without requiring additional cleaning.

8. Reagents

- 8.1 Analytical reagent grade chemicals shall be used in this practice for preparing all solutions.⁸
- 8.2 Use of triply Triply distilled water from coupled all-glass stills is recommended. or water from a high-quality commercial purification unit capable of achieving Total Oxidizable Carbon (T.O.C.) content below 5 ppb should be used. Type II reagent water as specified in ASTM Specification D1193 is also considered to be of sufficient quality for use in preparing solutions and 96 volume % ethanol.

Note 6—High-purity water is commercially available from some suppliers. Such water, labelled HPLC (high-pressure liquid chromatography) grade, is usually sufficiently free of impurities to be used in this practice.

9. Preparation of dosimeters

- 9.1 Dosimetric solutions may contain any concentration of CB. For practical reasons, only a few characteristic formulations have been thoroughly characterized. Table 1 lists these typical formulations in terms of CB concentrations and radiation chemical yields pertaining to these concentrations.
- 9.2 Prepare 96 volume % aqueous ethanol first by adding absolute ethanol into a volumetric flask containing the appropriate amount of water. (Warning—Ethanol is flammable.) Use this aqueous ethanol for making the dosimetric solutions of the desired concentrations by adding it into volumetric flasks containing appropriate amounts of CB. Store the dosimetric solution in the dark. (Warning—Chlorobenzene is toxic and a skin irritant. Appropriate precaution should be taken to avoid contact with the solution during preparation and analysis of the dosimeters. Used solutions should be disposed of as hazardous waste.)
- 9.3 Fill the dosimeter ampoules with the dosimetric solution. Bubble the solution in the ampoule with nitrogen for about 1 min at about 1 bubble per second through a 1-mm capillary. Flame-seal immediately after bubbling. Exercise care to avoid depositing solution in the ampoule neck. Store dosimeters in the dark.

Note 7—To minimize the removal of the vapor above the dosimetic solution in the ampoules, the nitrogen is saturated with the vapors of the dosimetric solution by passing it through ECB solution of the same composition before the bubbling of the dosimeter ampoules.

10. Calibration of the mercuric nitrate solution

- 10.1 The dosimeter measurement procedure is based on the titration of chloride ions formed by irradiation. Free chloride is precipitated with mercuric ions as insoluble $HgCl_2$, where-upon the excess of Hg^{2+} ions gives a violet-red coloration with the indicator diphenylcarbazone in acid medium (2119).
- 10.2 Prepare approximately 5×10^{-4} mol dm⁻³ Hg(NO₃)₂ in acidic aqueous ethanol. First dissolve an appropriate amount of Hg(NO₃)₂ in water acidified with sufficient HNO₃ to attain the concentration of the acid in the final solution, 0.05 mol dm⁻³. (**Warning**—Mercuric (II) nitrate is highly toxic. Acute exposure of skin and mucous membranes produces violent corrosive effects. Chronic exposure causes many pathological changes. Appropriate precautions should be exercised in handling it. Used solutions should be disposed of as hazardous waste. Hazards of mercury poisoning can be avoided by using some of the alternative readout methods described in Annex A2 and Table A3.1 in Annex A3.)

⁸ Reagent specifications are available from the American Chemical Society, 1115 16th Street, NW, Washington, DC 20036, USA.