Analysis: Dissolve the *Sample* with 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, preferably with a magnetic stirrer, add 25 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium gluceptate ($C_{14}H_{26}CaO_{16}$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 490.4 mg/mM
W = *Sample* weight (mg)

Acceptance criteria: 95.0%–102.0% on the dried basis

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩
  Standard: 1.0 mL of 0.020 N hydrochloric acid
  Sample: 1.0 g
  Acceptance criteria: NMT 0.07%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩
  Standard: 1.0 mL of 0.020 N sulfuric acid
  Sample: 2.0 g
  Acceptance criteria: NMT 0.05%
- **HEAVY METALS** ⟨**231**⟩
  Test preparation: Dissolve 1 g in 25 mL of water.
  Acceptance criteria: NMT 20 ppm
- **REDUCING SUGARS**
  Sample: 0.50 g
  Analysis: Dissolve the *Sample* in 10 mL of hot water, add 2 mL of 3 N hydrochloric acid, boil for about 2 min, and cool. Add 5 mL of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 mL, and filter. Add 5 mL of the clear filtrate to 2 mL of alkaline cupric tartrate TS, and boil for 1 min.
  Acceptance criteria: No red precipitate is formed immediately.

SPECIFIC TESTS

- **pH** ⟨**791**⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: 6.0–8.0
- **LOSS ON DRYING** ⟨**731**⟩
  (See *Thermal Analysis* ⟨891⟩.)
  [NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity. Weight loss occurring at temperatures above about 160°, indicative of decomposition, is not to be interpreted as *Loss on Drying*.]
  Sample: 10–25 mg
  Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat the specimen under test at a rate of 5°/min in an atmosphere of nitrogen, at a flow rate of 40 mL/min. Record the thermogram to 150°.
  Acceptance criteria: See *Table 1*.

Table 1

Form	Loss on Drying (%)
Anhydrous	NMT 1.0
$2H_2O$ (dihydrate)	NMT 6.9
$3\frac{1}{2}H_2O$	NMT 11.4

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label to indicate whether hydrous or anhydrous; if hydrous, label to indicate also the degree of hydration.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Calcium Gluceptate RS

Calcium Gluceptate Injection

» Calcium Gluceptate Injection is a sterile solution of Calcium Gluceptate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of calcium (Ca).

Packaging and storage—Preserve in tight, single-dose containers, preferably of Type I or Type II glass.

Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards ⟨11⟩**—**
USP Calcium Gluceptate RS
USP Endotoxin RS

Identification—

A: *Infrared Absorption* ⟨197K⟩—Prepare the test specimen as follows. Transfer 5 mL of Injection to a separator, add 10 mL of chloroform, shake, and allow the layers to separate. Draw off and discard the chloroform layer, and repeat the extraction with a second 10-mL portion of chloroform. Drain the water layer into a small beaker, evaporate to dryness, and dry in vacuum at 60° for 16 hours.

B: A dilution of the Injection with water (1 in 5) responds to the tests for *Calcium* ⟨191⟩.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.32 USP Endotoxin Unit per mg of calcium gluceptate.

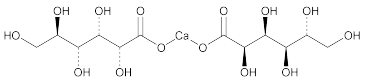
pH ⟨791⟩: between 5.6 and 7.0.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—To an accurately measured volume of Injection, equivalent to about 45 mg of calcium, add 2 mL of 3 N hydrochloric acid and 148 mL of water. While stirring, preferably with a magnetic stirrer, add about 15 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

Calcium Gluconate



$C_{12}H_{22}CaO_{14}$ 430.37

$C_{12}H_{22}CaO_{14} \cdot H_2O$ 448.39

D-Gluconic acid, calcium salt (2:1);
Calcium D-gluconate (1:2) [299-28-5].
Monohydrate [18016-24-5].

DEFINITION

Calcium Gluconate is anhydrous or contains one molecule of water of hydration. The anhydrous form contains NLT 98.0% and NMT 102.0% of calcium gluconate ($C_{12}H_{22}CaO_{14}$), calculated on the dried basis. The monohydrate form contains NLT 99.0% and NMT 101.0% of calcium gluconate monohydrate ($C_{12}H_{22}CaO_{14} \cdot H_2O$) where labeled as intended for use in preparing injectable dosage forms, and NLT 98.5% and NMT 102.0% of calcium gluconate monohydrate ($C_{12}H_{22}CaO_{14} \cdot H_2O$) where labeled as not intended for use in preparing injectable dosage forms.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨**191**⟩**:** A 20 mg/mL solution meets the requirements.
- **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Calcium Gluconate, heating in a water bath at 60°, if necessary, to dissolve

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent.* Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution.*

ASSAY

- **PROCEDURE**

Sample: 800 mg of Calcium Gluconate

Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium gluconate ($C_{12}H_{22}CaO_{14}$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mM/mL)
F = equivalency factor, 430.4 mg/mM
W = *Sample* weight (mg)

Acceptance criteria: Anhydrous form, 98.0%–102.0% on the dried basis; monohydrate form, 99.0%–101.0% where labeled as intended for use in preparing injectable dosage forms; and monohydrate form, 98.5%–102.0% where labeled as not intended for use in preparing injectable dosage forms

IMPURITIES

- **ARSENIC,** *Method I* ⟨**211**⟩

Test preparation: Dissolve 1.0 g in a mixture of 10 mL of hydrochloric acid and 20 mL of water, and dilute with water to 55 mL.

Analysis: Proceed as directed in the chapter, except to omit the addition of 20 mL of 7 N sulfuric acid.

Acceptance criteria: NMT 3 ppm

- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩**:** A 1.0-g portion shows no more chloride than corresponds to 0.07 mL of 0.020 N hydrochloric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 1.0-g portion shows no more chloride than corresponds to 1 mL of 0.020 N hydrochloric acid (0.07%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩**:** A 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 0.1 mL of 0.020 N sulfuric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 1 mL of 0.020 N sulfuric acid (0.05%).
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm; NMT 20 ppm where Calcium Gluconate is labeled as not intended for use in the preparation of injectable dosage forms
- **LIMIT OF IRON**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

Standard solutions: 0.2, 0.4, and 1.0 µg/mL of iron, prepared as follows. Separately transfer 2.0, 4.0, and 10.0 mL of *Standard Iron Solution*, prepared as directed under *Iron* ⟨241⟩, to individual 100-mL volumetric flasks, each containing 1.37 g of calcium chloride, previously tested and shown to contain less than 5 ppm of iron, and dilute with 2 N hydrochloric acid to volume.

Sample solution: Transfer 1.0 g of Calcium Gluconate to a 100-mL quartz glass flask. Add 20 mL of 12 N nitric acid, and heat to boiling until fumes are evolved. Add 0.5 mL of 30% hydrogen peroxide, and heat again until fumes are evolved. Repeat this process until the volume is reduced to about 5 mL. Cool, add 1.0 mL of perchloric acid, and heat to boiling. [**CAUTION—**Do not heat above 190° or evaporate to dryness because of danger of explosion.] Transfer this solution to a 25-mL volumetric flask, and dilute with 2 N hydrochloric acid to volume.

Blank solution: Use 0.34 g of calcium chloride, previously tested and shown to contain less than 5 ppm of iron, instead of Calcium Gluconate, and prepare as directed under *Sample solution.*

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 248.3 nm

Lamp: Iron hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*, using the *Blank solution* as the blank and making deuterium background corrections. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of iron, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in µg/mL, of iron in the *Sample solution.*

Calculate the concentration of iron, in ppm, in the portion of Calcium Gluconate taken:

$$\text{Result} = (C \times V)/W$$

C = concentration of iron in the *Sample solution* obtained from the regression equation (µg/mL)
V = volume of *Sample solution* (mL)
W = weight of Calcium Gluconate taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 5 ppm

- **LIMIT OF MAGNESIUM AND ALKALI METALS**

[NOTE—Calcium Gluconate labeled as not intended for use in preparing injectable dosage forms is exempt from this requirement.]

Sample: 1.0 g

Analysis: Dissolve completely the *Sample* in 100 mL of boiling water. Add 10 mL of ammonium chloride TS, 1 mL of ammonium hydroxide, and 50 mL of hot (maintained at 70°–80°) ammonium oxalate TS. Allow to stand for 4 h, dilute with water to 200 mL, and filter. Evaporate 100 mL of the filtrate to dryness, and ignite to constant weight.

Acceptance criteria: NMT 0.4%: The weight of the residue does not exceed 2 mg.

- **LIMIT OF PHOSPHATE**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

Standard stock solution 1: 0.716 mg/mL of monobasic potassium phosphate

Standard stock solution 2: Dilute 1.0 mL of *Standard stock solution 1* with water to 100 mL.

Standard solution: Dilute 2.0 mL of *Standard stock solution 2* with water to 100 mL.

Sample stock solution: To 10.0 g of Calcium Gluconate add 90 mL of hot water (70°–80°), and heat to boiling, with swirling, for 10 s to obtain a clear solution.

Sample solution: Dilute 1 mL of the hot *Sample stock solution* with water to 100 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

To the *Standard solution* and *Sample solution* add 4 mL of sulfomolybdic acid TS, and mix. To both solutions add 0.1 mL of a freshly prepared mixture of 3 N hydrochloric acid and stronger acid stannous chloride TS (10:1), and mix.

Acceptance criteria: NMT 0.01%: After 10 min any color in the *Sample solution* is not more intense than that in the *Standard solution*.

- **LIMIT OF OXALATE**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

[NOTE—Use deionized water where water is indicated.]

Mobile phase: 0.0017 M sodium bicarbonate and 0.0018 M sodium carbonate in water

Solution A: 0.0125 M sulfuric acid in water

Solution B: Dilute 1 mL of hydrochloric acid with water to 1200 mL.

Standard solution: 1.5 µg/mL of sodium oxalate in *Solution B*

Sample solution: 20 mg/mL of Calcium Gluconate in *Solution B*. Sonicate if necessary.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Ion chromatography

Detector: Conductance

Columns

Analytical: 4-mm × 25-cm; 15-µm packing L12

Guard: 4-mm × 5-cm; 15-µm packing L12

Anion suppressor: Micromembrane anion suppressor column connected in series with the guard and analytical columns. The anion suppressor column is equipped with a micromembrane that separates the *Mobile phase* from the *Solution A* flowing countercurrent to the *Mobile phase* at a rate of about 7 mL/min.

[NOTE—Condition the system for about 15 min with *Mobile phase* at a flow rate of 2 mL/min.]

Flow rate: 2 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2500 theoretical plates

Tailing factor: NMT 1.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxalate in the portion of Calcium Gluconate taken:

$$\text{Result: } (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F \times 100$$

r_U = peak response for oxalate from the *Sample solution*
r_S = peak response for oxalate from the *Standard solution*
C_S = concentration of sodium oxalate in the *Standard solution* (µg/mL)
C_U = concentration of Calcium Gluconate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxalate, 88.03
M_{r2} = molecular weight of sodium oxalate, 134.00
F = conversion factor, 0.001 mg/µg

Acceptance criteria: NMT 0.01%

- **REDUCING SUBSTANCES**

Sample: 1.0 g of Calcium Gluconate

Blank: 20 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 0.1 N iodine VS

Back titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 20 mL of hot water, cool, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with the *Back titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back titrant* volume consumed by the *Blank* (mL)
V_S = *Back titrant* volume consumed by the *Sample* (mL)
N = *Back titrant* normality (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° for 16 h: the anhydrous form loses NMT 3.0% of its weight; the monohydrate form, where labeled as intended for use in preparing injectable dosage forms, loses NMT 1.0% of its weight, and where labeled as not intended for use in preparing injectable dosage forms, loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is anhydrous or monohydrate. Where the quantity of calcium gluconate is indicated in the labeling of any solution containing Calcium Gluconate, this shall be understood to be in terms of anhydrous calcium gluconate ($C_{12}H_{22}CaO_{14}$).

Calcium Gluconate intended for use in preparing injectable dosage forms is so labeled. Calcium Gluconate not intended for use in preparing injectable dosage forms is so labeled; in addition, it may be labeled also as intended for use in preparing oral dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS

Calcium Gluconate Injection

» Calcium Gluconate Injection is a sterile solution of Calcium Gluconate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of total Ca. The calcium is in the form of calcium gluconate, except that a small amount may be replaced with an equal amount of calcium in the form of Calcium Saccharate, or other suitable calcium salts, for the purpose of stabilization. It may contain sodium hydroxide added for adjustment of the pH.

Injection intended for veterinary use only may be prepared from Calcium Gluconate solubilized with Boric Acid, or from Glyconolactone, Boric Acid, and Calcium Carbonate.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

Labeling—Label the Injection to indicate its content, if any, of added calcium salts, calculated as percentage of calcium in the Injection. The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL. The labeling indicates that the Injection must be clear at the time of use, and that if crystallization has occurred, warming may redissolve the precipitate. Injection intended for veterinary use only is so labeled. If Injection contains Boric Acid, it is so labeled.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS
USP Potassium Gluconate RS

Identification—

A: A volume of Injection diluted, if necessary, with water to obtain a test solution of calcium gluconate (1 in 100) responds to Identification test *B* under *Calcium Gluconate*.

B: A dilution of the Injection with water (1 in 5) responds to the tests for *Calcium* ⟨191⟩.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.17 USP Endotoxin Unit per mg of calcium gluconate.

pH ⟨791⟩: between 6.0 and 8.2, except that in the case where it is labeled as intended for veterinary use only and as containing boric acid, it is between 2.5 and 4.5.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—To an accurately measured volume of Injection, equivalent to about 500 mg of calcium gluconate, add 2 mL of 3 N hydrochloric acid, and dilute with water to 150 mL. While stirring, preferably with a magnetic stirrer, add about 20 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

Calcium Gluconate Tablets

DEFINITION
Calcium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of calcium gluconate ($C_{12}H_{22}CaO_{14}$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨**191**⟩
Sample stock solution: A warm, filtered solution in water, equivalent to 100 mg/mL of calcium gluconate from powdered Tablets
Sample solution: Equivalent to 20 mg/mL of calcium gluconate from a dilution of the *Sample stock solution*
Acceptance criteria: Meet the requirements

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**
Standard solution: 10 mg/mL of USP Potassium Gluconate RS
Sample solution: Equivalent to 10 mg/mL of calcium gluconate from a dilution of the *Sample stock solution* obtained from *Identification* test *A*
Chromatographic system
(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Develop until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent.* Heat the plate at 110° for about 10 min.
Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution.*

ASSAY

• **PROCEDURE**
Sample: A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 500 mg of calcium gluconate
Blank: Proceed as directed in the *Analysis*, without the *Sample*.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Indicator: Hydroxy naphthol blue, 300 mg
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a suitable crucible. Ignite, gently at first, until free from carbon. Cool the crucible. Add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to achieve complete solution. Transfer the solution to a suitable container, and add about 150 mL of water. While stirring, preferably with a magnetic stirrer, add 20 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide, then add the *Indicator*. Continue the titration to a blue endpoint. Perform a *Blank* determination.