User Guide

Calcium
Ion Selective
Electrode

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This publication supersedes all previous publications on this subject.

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Introduction

This user guide contains information on the preparation, operation and maintenance for the calcium ion selective electrode (ISE). General analytical procedures, electrode characteristics and electrode theory are also included in this user guide. Calcium electrodes measure free calcium ions in aqueous solutions quickly, simply, accurately and economically.

Technical Support Chemists can be consulted for assistance and troubleshooting advice. Within the United States call 1.800.225.1480 and outside the United States call 978.232.6000 or fax 978.232.6031. In Europe, the Middle East and Africa, contact your local authorized dealer. For the most current contact information, visit www.thermo.com/contactwater.

For the latest application and technical resources for Thermo Scientific Orion products, visit www.thermo.com/waterapps.

Calcium ionplus® Sure-Flow® Plastic Membrane Combination ISE, Cat. No. 9720BNWP

The calcium combination electrode has the sensing and reference half-cells built into one electrode, which decreases the amount of required solutions and reduces waste. The built-in Sure-Flow reference junction prevents electrode clogging and provides fast and stabile readings. The calcium combination electrode is available with a waterproof BNC connector, Cat. No. 9720BNWP. Electrodes with a waterproof BNC connector can be used on any ISE or mV meter with a BNC connection.

Calcium Plastic Membrane Half-Cell ISE, Cat. No. 9320BN

The calcium half-cell electrode must be used with the single junction reference electrode, Cat. No. 900100. The calcium half-cell electrode is available with a BNC connector, Cat. No. 9320BN.

Required Equipment

1. Thermo Scientific Orion ISE meter, such as the 4-Star pH/ISE meter or 5-Star pH/ISE/DO/conductivity meter; equivalent ISE meter; or mV meter with a 0.1 mV resolution.

   Calcium electrodes can be used on any ISE or mV meter with a BNC connection. The electrodes can also be used on meters with a variety of inputs when an adapter cable is used. Visit www.thermo.com/water for details.

2. Thermo Scientific Orion calcium electrode.

   The 9320BN calcium half-cell electrode requires a separate reference electrode, Cat. No. 900100.

3. Magnetic stirrer or Thermo Scientific Orion stirrer probe, Cat. No. 096019. The stirrer probe can be used with 3-Star, 4-Star and 5-Star benchtop meters.

4. Volumetric flasks, graduated cylinders and beakers. Plastic labware is required for low level calcium analysis.

5. Distilled or deionized water.

6. Calcium electrode filling solution.

   Use Optimum Results™ A filling solution, Cat. No. 900061, for the 9720BNWP calcium combination electrode.

   Use 4 M KCl saturated with Ag/AgCl filling solution, Cat. No. 900011, for the single junction reference electrode that is used with the 9320BN calcium half-cell electrode.

7. 0.1 M $CaCl_2$ calcium calibration standard, Cat. No. 922006 or 100 ppm calcium as $CaCO_3$ calibration standard, Cat. No. 923206.

   1000 ppm calcium as Ca^{+2} solution – Add 25 mL of the 0.1 M $CaCl_2$ calcium standard to a 100 mL volumetric flask, dilute to the mark with distilled water and mix the solution.

   1000 ppm calcium as $CaCO_3$ solution – Add 10 mL of the 0.1 M $CaCl_2$ calcium standard to a 100 mL volumetric flask, dilute to the mark with distilled water and mix the solution.

8. Calcium ionic strength adjuster (ISA), Cat. No. 932011. ISA provides a constant background ionic strength for samples and standards.

Serial Dilutions

Serial dilution is the best method for the preparation of standards. Serial dilution means that an initial standard is diluted, using volumetric glassware, to prepare a second standard solution. The second standard is similarly diluted to prepare a third standard, and so on, until the desired range of standards has been prepared.

1. **To prepare a 10^{-2} M standard (400.8 ppm as Ca^{+2} and 1000.9 as $CaCO_3$) –** Pipet 10 mL of the 0.1 M standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

2. **To prepare a 10^{-3} M standard (40.08 ppm as Ca^{+2} and 100.09 as $CaCO_3$) –** Pipet 10 mL of the 10^{-2} M standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

3. **To prepare a 10^{-4} M standard (4.008 ppm as Ca^{+2} and 10.009 as $CaCO_3$) –** Pipet 10 mL of the 10^{-3} M standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

To prepare standards with a different concentration use the following formula:

$$\mathbf{C_1 * V_1 = C_2 * V_2}$$

C_1 = concentration of original standard
V_1 = volume of original standard
C_2 = concentration of standard after dilution
V_2 = volume of standard after dilution

For example, to prepare 100 mL of a 100 ppm calcium standard from a 4008 ppm calcium standard:

C_1 = 4008 ppm calcium

V_1 = unknown

C_2 = 100 ppm calcium

V_2 = 100 mL

4008 ppm * V_1 = 100 ppm * 100 mL

V_1 = (100 ppm * 100 mL) / 4008 ppm = 2.5 mL

Electrode Setup

9720BNWP Calcium Combination Electrode Preparation

Note: *Do not to touch the sensing membrane or reference pellet during the electrode assembly.*

1. Remove the sensing module from the vial and save the vial for storage. Make sure that both O-rings are in place on the module. Remove the electrode handle from the box.

2. Unscrew the electrode cap. Slide the cap and spring down the electrode cable.

3. Hold the outer body sleeve and gently push the inner stem through the outer body. Slide the outer body sleeve down the electrode cable until it is beyond the inner stem.

4. Grasp the middle of the inner stem without touching the reference pellet. If a red storage tip is connected to the inner stem, unscrew it and save it for storage.

5. Screw the sensing module into the stem until it stops and the module is flush against the stem. Tighten the module an additional one-quarter turn. The module should be firmly attached to the stem. Do not overtighten the module.

6. Hold the electrode cable and slide the outer body, spring and cap over the inner stem.

7. Grasp the outer body sleeve, without touching the sensing membrane, and gently screw the cap onto the inner stem while pulling on the cable. Stop when an opposite force is felt. Do not overtighten or continue to turn the cap. The cap will not completely stop. If the inner body turns at all, the cap is too tight. Remove the cap and reassemble.

8. Press on the top of the cap with your thumb to make sure that the electrode has a smooth flushing motion and the outer body sleeve returns to its original position.

9. Install the flip spout cap onto the Optimum Results A filling solution bottle and lift the flip spout to a vertical position. Insert the spout into the electrode fill hole and add a small amount of filling solution to the reference chamber.

10. Hold the electrode body and use your thumb to push down on the electrode cap to allow a few drops of filling solution to drain out of the electrode. Release the electrode cap.

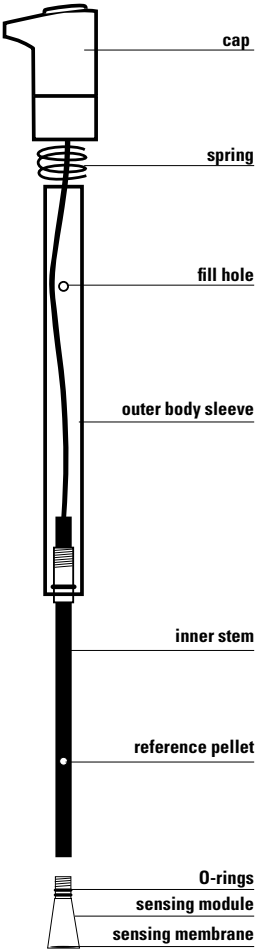
11. If the sleeve does not return to its original position, add filling solution and repeat step 10 until the sleeve returns to its original position.

12. Add filling solution to the electrode up to the fill hole.

13. Rinse the electrode with distilled water and soak it in a 100 ppm or 10^{-2} M calcium standard for 1 to 2 hours prior to use.

Note: *Add filling solution each day before using the electrode. The filling solution level should be at least one inch above the level of sample in the beaker to ensure a proper flow rate. The fill hole should always be open when taking measurements.*

Figure 1
9720BNWP Calcium Combination Electrode



9320BN Calcium Half-Cell Electrode Preparation

Remove the sensing module from the vial and save the vial for electrode storage. Make sure that the rubber electrode washer on the sensing module is in place. See **Figure 2**. Screw the sensing module into the electrode body until the module is finger-tight. To ensure electrical continuity, shake the electrode down like a clinical thermometer. Rinse the calcium electrode with distilled water and then soak it in a 100 ppm or 10^{-2} M calcium standard for 1 to 2 hours prior to use.

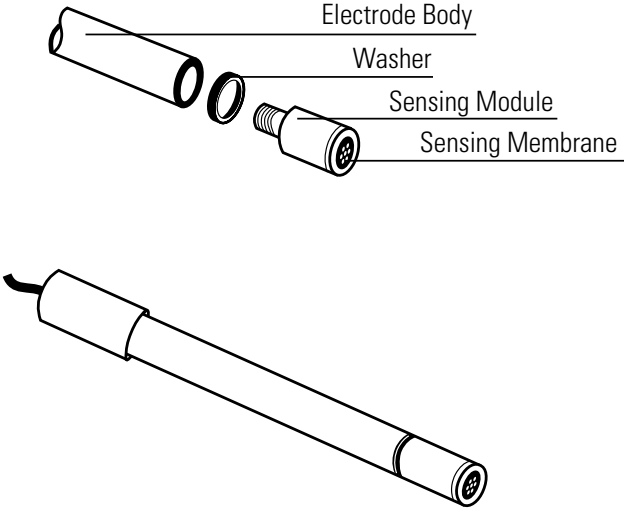
Note: *Do not immerse the electrode past the rubber electrode washer.*

900100 Single Junction Reference Electrode Preparation

Prepare the reference electrode according to the reference electrode user guide. Fill the reference electrode with 4 M KCl saturated with Ag/AgCl filling solution, Cat. No. 900011.

Note: *Do not use the filling solution that ships with the 900100 single junction reference electrode because it contains interferences for calcium measurements.*

Figure 2
9320BN Calcium Half-Cell Electrode



Checking Electrode Operation (Slope)

These are general instructions that can be used with most meters to check the electrode operation. Refer to the meter user guide for more specific information.

This procedure measures electrode slope. Slope is defined as the change in millivolts observed with every tenfold change in concentration. Obtaining the slope value provides the best means for checking electrode operation.

1. If the electrode has been stored dry, prepare the electrode as described in the **Electrode Preparation** section.

2. Connect the electrode to a meter with a mV mode. Set the meter to the mV mode.

3. Add 100 mL of distilled water and 2 mL of ISA into a 150 mL beaker. Stir the solution thoroughly.

4. Rinse the electrode with distilled water and place the electrode into the solution prepared in step 3.

5. Select either a 0.1 M or 1000 ppm calcium standard. Pipet 1 mL of the standard into the beaker and stir the solution thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

6. Pipet 10 mL of the same standard into the same beaker and stir the solution thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

7. There should be a 25 to 30 mV difference between the two millivolt readings when the solution temperature is between 20 to 25 °C. If the millivolt potential is not within this range, refer to the **Troubleshooting** section.

Measurement Units

Calcium concentration can be measured in moles per liter (M), parts per million (ppm) or any convenient concentration unit.

Table 1
Concentration Unit Conversion Factors

Moles/Liter (M)	ppm as Ca^{+2}	ppm as $CaCO_3$
1.0	40080	100089
10^{-1}	4008	10008.9
10^{-2}	400.8	1000.89
10^{-3}	40.1	100.089
10^{-4}	4.0	10.0089

Sample Requirements

All samples must be aqueous and must not contain organic solvents. Contact Technical Support for information on using the electrode for specific applications.

The solution temperature must be less than 40 °C.

Samples and standards should be at the same temperature. A 1 °C difference in temperature for a 10^{-3} M calcium solution will give rise to about a 4% error when using the 9320BN calcium half-cell electrode and about a 1.2% error when using the 9720BNWP calcium combination electrode.

Interferences should be absent from all samples. See the **Interferences** section for a list of possible interferences.

In all analytical procedures, ISA must be added to all samples and standards before measurements are taken.

Measuring Hints

- Stir all standards and samples at a uniform, moderate rate. Place a piece of insulating material, such as Styrofoam or cardboard, between the magnetic stir plate and beaker to prevent measurement errors from the transfer of heat to the sample.

- Always use freshly prepared standards for calibration.

- Always rinse the electrode with distilled water between measurements and shake the electrode to remove the water and prevent sample carryover. Do not wipe or rub the electrode sensing module.

- Store the calcium electrode in a 10^{-2} M or 100 ppm calcium standard between measurements.

- The 9320BN calcium half-cell electrode should be immersed in standards and samples to approximately half the length of the calcium module. Do not immerse the calcium electrode past the electrode washer. Immerse the reference electrode to the same depth as the calcium electrode.

- Allow all standards and samples to reach the same temperature for precise measurements.

- Verify the electrode calibration every two hours by placing the electrode in a fresh aliquot of the least concentrated standard used for calibration. If the value has changed by more than 2%, recalibrate the electrode.

- After immersing the electrode in a solution, check the electrode sensing surface for air bubbles and remove air bubbles by reimmersing the electrode in the solution and gently tapping it.

- For high ionic strength samples, prepare standards with a background composition similar to the sample.

- The fill hole cover must be open during measurements to ensure a uniform flow of filling solution.

- If the combination electrode is used and the electrode is used in dirty or viscous samples or the electrode response becomes sluggish, empty the electrode completely, hold the junction open and flush the junction with distilled water. Empty any water from the electrode and refill it with fresh filling solution. Press down on the electrode cap to let a few drops of the filling solution flow out of the electrode and then replenish any lost solution.

Electrode Storage

Calcium Combination Electrode Storage, Cat. No. 9720BNWP

For storage between measurements and up to three days, store the electrode in a 10^{-2} M or 100 ppm calcium standard. The filling solution inside the electrode should not be allowed to evaporate, as crystallization will result.

For storage longer than one week, drain the electrode, flush the reference chamber with distilled water, disassemble the electrode and store the sensing module in the glass vial.

1. Grasp the outer body sleeve and unscrew the electrode cap. Slide the cap and spring assembly down the electrode cable.

2. Push the inner stem of the electrode handle out through the outer electrode sleeve, exposing the sensing module.

3. Rinse the inner stem and module well with distilled water. Gently blot dry to prevent damaging the sensing module.

4. Carefully unscrew the sensing module from the inner stem, taking care not to touch the sensing membrane.

5. Place the calcium sensing module in the glass vial until it is needed again. Gently blot dry the inside of the inner stem and O-ring area, reassemble the electrode handle without the module and store it dry.

Calcium Half-Cell Electrode Storage, Cat. No. 9320BN

The calcium half-cell electrode should be rinsed thoroughly with distilled water and stored a 10^{-2} M or 100 ppm calcium standard. When storing the electrode for more than three days, rinse the calcium half-cell electrode thoroughly with distilled water, shake the electrode dry, disassemble the electrode and store the sensing module in the glass vial.

Single Junction Reference Electrode Storage, Cat. No. 900100

The single junction reference electrode may be stored in the filling solution, Cat. No. 900011, between sample measurements and up to one week. The filling solution inside the electrode should not be allowed to evaporate, as crystallization will result.

For storage longer than one week, drain the reference electrode, flush the inside with distilled water and store the electrode dry.

Electrode Maintenance

Cleaning the Calcium Sensing Module

If the electrode is exposed to high levels of interfering ions, it may drift and become sluggish in response. When this happens, restore normal performance by soaking the electrode for an hour in distilled water, emptying the old filling solution, filling the electrode with fresh filling solution and then soaking the electrode for a few hours a 10^{-2} M or 100 ppm calcium standard. If soaking the electrode does not restore normal electrode performance, replace the calcium sensing module.

Calcium Combination Electrode and Double Junction Reference Electrode Flushing

If the area between the electrode outer body and inner cone becomes clogged with sample or precipitate, flush the area with filling solution or distilled water.

1. Hold the electrode body with one hand and use your thumb to push down on the electrode cap to drain all of the filling solution out of the electrode.

2. Fill the electrode with distilled water and then push down on the cap until all the water is drained from the chamber. Repeat this procedure until all of the sample or precipitate is removed from the electrode.

3. Fill the electrode with fresh filling solution up to the fill hole. Push down on the cap to allow a few drops of filling solution to drain out of the electrode and then replenish the lost filling solution.

4. Rinse the electrode with distilled water and soak it in a 10^{-2} M or 100 ppm calcium standard for 1 to 2 hours.

Replacing the Calcium Sensing Module

The sensing membrane of plastic membrane electrodes will wear over time, indicated by low slope values, drift, poor reproducibility and loss of response in low level samples. The electrode response can be restored by replacing the sensing module. Each sensing module will last about six months with normal laboratory use, but the actual lifespan of the sensing module will depend on the type of samples that are measured.

For the 9720BNWP calcium combination electrode, use the 97 series calcium module, Cat. No. 972001.

Drain the electrode and flush the reference chamber with distilled water. Hold the outer body sleeve and unscrew the electrode cap. Slide the cap and spring assembly down the electrode cable. Push the inner stem of the electrode handle out through the outer electrode sleeve, exposing the sensing module. Rinse the inner stem and module well with distilled water. Gently blot dry to prevent damaging the sensing module. Carefully unscrew the sensing module from the inner stem and dispose of the old sensing module. Obtain a new 97 series calcium module, Cat. No. 972001, and refer to the **9720BNWP Calcium Combination Electrode Preparation** section for detailed instructions on assembling the electrode.

For the 9320BN calcium half-cell electrode, use the 93 series calcium module, Cat. No. 932001.

Rinse the electrode with distilled water. Carefully unscrew the sensing module from the electrode and dispose of the old sensing module. Obtain a new 93 series calcium module, Cat. No. 932001, and refer to the **9320BN Calcium Half-Cell Electrode Preparation** section for detailed instructions on assembling the electrode.

Analytical Techniques

A variety of analytical techniques are available to the analyst. The following is a description of these techniques.

Direct Calibration is a simple procedure for measuring a large number of samples. Only one meter reading is required for each sample. Calibration is performed using a series of standards. The concentration of the samples is determined by comparison to the standards. ISA is added to all solutions to ensure that samples and standards have similar ionic strength.

Low Level Calibration is a similar to the direct calibration technique. This method is recommended when the expected sample concentration is less than 0.4 ppm or 10^{-5} M calcium. A minimum three point calibration is recommended to compensate for the electrode's non-linear response at these concentrations. A special calibration standard preparation procedure is the best means of preparing low level calibration standards.

Incremental Techniques provide a useful method for measuring samples, since a calibration is not required. The different incremental techniques are described below. They can be used to measure the total concentration of a specific ion in the presence of a large (50 to 100 times) excess of complexing agents. As in direct calibration, any convenient concentration unit can be used.

Known Addition is useful for measuring dilute samples, checking the results of direct calibration (when no complexing agents are present), or measuring the total concentration of an ion in the presence of an excess complexing agent. The electrode is immersed in the sample solution and an aliquot of a standard solution containing the measured species is added to the sample. From the change in potential before and after the addition, the original sample concentration is determined.

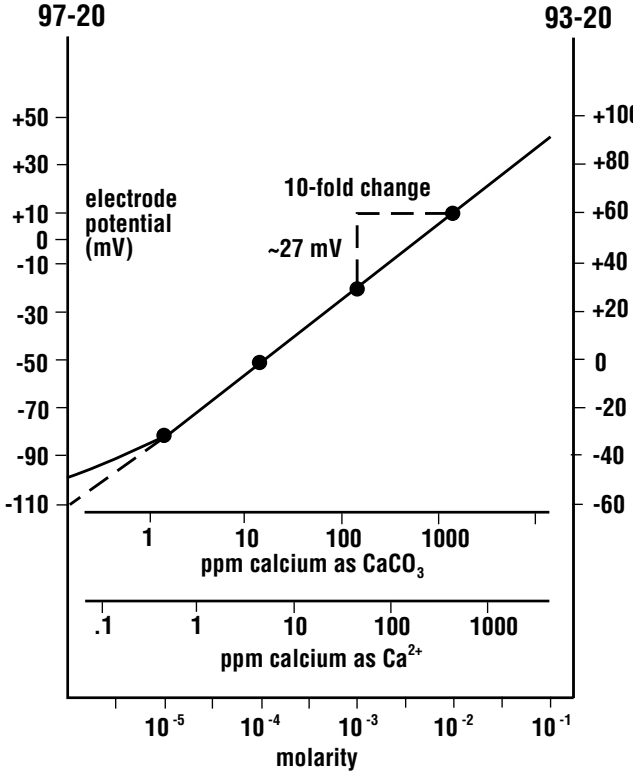
Titrations are quantitative analytical techniques for measuring the concentration of a species by incremental addition of a reagent (titrant) that reacts with the sample species. Sensing electrodes can be used for determination of the titration end point. Ion selective electrodes are useful as end point detectors, because they are unaffected by sample color or turbidity. Titrations are approximately 10 times more precise than direct calibration, but are more time-consuming.

Direct Calibration Technique

Typical Direct Calibration Curve

In the direct calibration procedure, a calibration curve is constructed either in the meter memory or on semi-logarithmic paper. Electrode potentials of standard solutions are measured and plotted on the linear axis against their concentrations on the log axis. In the linear regions of the curves, only two standards are needed to determine a calibration curve. In non-linear regions, more points must be taken. These direct calibration procedures are given for concentrations in the region of linear electrode response. Low level measurement procedures are given in a following section for measurements in the non-linear electrode region.

Figure 3
Typical Direct Calibration Curve



Direct Calibration Overview

The following direct measurement procedures are recommended for moderate to high level measurements. Samples must be in the linear range of the electrode – greater than 0.4 ppm or 10^{-5} M calcium. A two point calibration is sufficient, although more points can be used. When using an ISE meter, sample concentrations can be read directly from the meter. When using a mV meter, a calibration curve can be prepared on semi-logarithmic graph paper, or a linear regression (against logarithmic concentration values) can be performed using a spreadsheet or graphing program.

Calibration Hints

- Standard concentrations should bracket the expected sample concentrations.
- Always add 2 mL of ISA, Cat. No. 932011, per 100 mL of standard or sample.
- For high ionic strength samples that have an ionic strength of 0.1 M or greater, prepare standards with a background composition similar to that of the samples, or measure the samples using the known addition method.
- During calibration, measure the least concentrated standard first, and work up to the most concentrated standard.

Direct Calibration Setup

1. Prepare the electrode as described in the **Electrode Preparation** section. If using the 9720BNWP combination calcium electrode, fill the electrode with Cat. No. 900061. If using the 9320BN half-cell calcium electrode with the 900100 reference electrode, fill the reference electrode with 4 M KCl saturated with Ag/AgCl filling solution, Cat. No. 900011.
2. Connect the electrode to the meter.
3. Prepare at least two standards that bracket the expected sample range and differ in concentration by a factor of ten. Standards can be prepared in any concentration unit to suit the particular analysis requirement. See the **Serial Dilution** section for instructions on how to prepare standards. All standards should be at the same temperature as the samples. For details on temperature effects on electrode performance, refer to the **Temperature Effects** section.

Direct Calibration Procedure Using a Meter with an ISE Mode

Note: *See the meter user guide for more specific information.*

1. Add 100 mL of the less concentrated standard and 2 mL of ISA to a 150 mL beaker and stir the solution thoroughly.

2. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. Wait for a stable reading and adjust the meter to display the value of the standard, as described in the meter user guide.

3. Add 100 mL of the more concentrated standard and 2 mL of ISA to a second 150 mL beaker and stir the solution thoroughly.

4. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. Wait for a stable reading and adjust the meter to display the value of the second standard, as described in the meter user guide.

5. Record the resulting slope value. The slope should be between 25 and 30 mV when the standards are between 20 and 25 °C.

6. Add 100 mL of sample and 2 mL of ISA to a clean 150 mL beaker and stir the solution thoroughly.

7. Rinse the electrode with distilled water, blot it dry and place it into the sample. The concentration of the sample will be displayed on the meter.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Direct Calibration Procedure Using a Meter with a mV Mode

Note: *See the meter user guide for more specific information.*

1. Set the meter to the mV mode.

2. Add 100 mL of the less concentrated standard and 2 mL of ISA to a 150 mL beaker and stir the solution thoroughly.

3. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

4. Add 100 mL of the more concentrated standard and 2 mL of ISA to a second 150 mL beaker and stir the solution thoroughly.

5. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

6. Using semi-logarithmic graph paper, prepare a calibration curve by plotting the millivolt values on the linear axis and the standard concentration values on the logarithmic axis.

7. Add 100 mL of sample and 2 mL of ISA to a clean 150 mL beaker and stir the solution thoroughly.

8. Rinse the electrode with distilled water, blot it dry and place it into the beaker. When a stable reading is displayed, record the mV value.

9. Using the calibration curve prepared in step 6, determine the unknown concentration of the sample.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Small Volume Direct Calibration Technique

Take advantage of special design features available with the 9720BNWP ionplus combination calcium electrode to meet your measuring needs. Due to the Sure-Flow reference, this electrode is able to measure sample volumes as small as 5 mL using a modified direct measurement procedure. Because less solution volume is required, the chemical usage of calcium standards and ISA is reduced. This method is also convenient when making field measurements, since the 9720BNWP combination calcium electrode does not require a separate reference electrode. All samples should have a concentration greater than 1 ppm or 10^{-4} M calcium. A two point calibration is sufficient, although more points can be used. The following procedure recommends using 25 mL of sample. Smaller sample volumes can be used, as long as the final volume of solution is sufficient to cover the bottom of the electrode.

Calibration Hints

- Use the 9720BNWP ionplus combination calcium electrode.
- Standard concentrations should bracket the expected sample concentrations.
- Always keep the ratio of standard or sample to ISA at 50:1.
- For high ionic strength samples that have an ionic strength of 0.1 M or greater, prepare standards with a background composition similar to that of the samples, or measure the samples using the known addition method.
- During calibration, measure the least concentrated standard first, and work up to the most concentrated standard.
- Calibrate with the same volume of standard as the volume of sample that is available for analysis.

Small Volume Direct Calibration Setup

1. Prepare the 9720BNWP combination calcium electrode as described in the **Electrode Preparation** section and fill the electrode with Optimum Results A filling solution, Cat. No. 900061.

2. Connect the electrode to the meter.

3. Prepare at least two standards that bracket the expected sample range and differ in concentration by a factor of ten. Standards can be prepared in any concentration unit to suit the particular analysis requirement. See the **Serial Dilution** section for instructions on how to prepare standards. All standards should be at the same temperature as the samples. For details on temperature effects on electrode performance, refer to the **Temperature Effects** section.

Small Volume Direct Calibration Procedure Using a Meter with an ISE Mode

Note: *See the meter user guide for more specific information.*

1. Add 25 mL of the less concentrated standard and 0.5 mL of ISA to a 50 mL beaker and swirl the solution to mix.

2. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. Wait for a stable reading and adjust the meter to display the value of the standard, as described in the meter user guide.

3. Add 25 mL of the more concentrated standard and 0.5 mL of ISA to a second 50 mL beaker and swirl the solution to mix.

4. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. Wait for a stable reading and adjust the meter to display the value of the second standard, as described in the meter user guide.

5. Record the resulting slope value. The slope should be between 25 and 30 mV when the standards are between 20 and 25 °C.

6. Add 25 mL of sample and 0.5 mL of ISA to a clean 50 mL beaker and swirl the solution to mix.

7. Rinse the electrode with distilled water, blot it dry and place it into the sample. The concentration of the sample will be displayed on the meter.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Small Volume Direct Calibration Procedure Using a Meter with a mV Mode

Note: *See the meter user guide for more specific information.*

1. Set the meter to the mV mode.

2. Add 25 mL of the less concentrated standard and 0.5 mL of ISA to a 50 mL beaker and swirl the solution to mix.

3. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

4. Add 25 mL of the more concentrated standard and 0.5 mL of ISA to a second 50 mL beaker and swirl the solution to mix.

5. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

6. Using semi-logarithmic graph paper, prepare a calibration curve by plotting the millivolt values on the linear axis and the standard concentration values on the logarithmic axis.

7. Add 25 mL of sample and 0.5 mL of ISA to a clean 50 mL beaker and swirl the solution to mix.

8. Rinse the electrode with distilled water, blot it dry and place it into the beaker. When a stable reading is displayed, record the mV value.

9. Using the calibration curve prepared in step 6, determine the unknown concentration of the sample.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Low Level Calibration Technique

These procedures are for solutions that have a calcium concentration of less than 0.4 ppm or 10^{-5} M calcium. For solutions low in calcium but high in total ionic strength (greater than 10^{-1} M), perform the same procedure by preparing a calibrating solution with a composition similar to the sample.

Accurate results require that the following conditions be met:

- Prepare at least three calibration standards that bracket the expected sample concentration.
- Always use low level ISA for standards and samples.
- Plastic labware must be used for all low level calcium measurements.
- Adequate time must be allowed for electrode stabilization. Longer response time will be needed at low level measurements.
- Stir all standards and samples at a uniform rate.

Low Level Setup

1. Prepare the electrode as described in the **Electrode Preparation** section.
2. Connect the electrode to the meter. Set the meter to the mV mode.
3. Prepare the low level ISA by pipetting 25 mL of the ISA, Cat. No. 932011, into a 100 mL volumetric flask and diluting to the mark with distilled water. Use low level ISA for low level measurements only.
4. Select a standard solution. Use either a 10 ppm calcium as $CaCO_3$ standard or a 10^{-4} M $CaCl_2$ calcium standard.

Low Level Calibration and Measurement

1. Add 100 mL of distilled water and 1 mL of low level ISA to a 150 mL beaker.

2. Rinse the electrode with distilled water, blot it dry and place it into the beaker. Stir the solution thoroughly.

3. Add increments of the 10 ppm or 10^{-4} M calcium standard to the beaker using the steps outlined in **Table 2**. Record the stable millivolt reading after each increment.

4. On semi-logarithmic paper, plot the concentration (log axis) against the millivolt potential (linear axis). Prepare a new calibration curve with fresh standards each day.

5. Measure 100 mL of sample and 1 mL of low level ISA and pour the solutions into a clean 150 mL beaker. Rinse the electrode with distilled water, blot it dry and place the electrode into the sample.

6. Stir the solution thoroughly. When a stable reading is displayed, record the mV value.

7. Determine the sample concentration corresponding to the measured potential from the low level calibration curve.

Table 2
Calibration Curve For Low Level Calibrations
Additions of standard to 100 mL distilled water and 1 mL low level ISA solution.

Step	Pipet Size	Volume Added	Concentration ppm	Concentration M
1	1 mL	0.1 mL	0.01	1.0×10^{-7}
2	1 mL	0.1 mL	0.02	2.0×10^{-7}
3	1 mL	0.2 mL	0.04	3.9×10^{-7}
4	1 mL	0.2 mL	0.06	6.0×10^{-7}
5	1 mL	0.4 mL	0.10	9.8×10^{-7}
6	2 mL	2.0 mL	0.29	2.9×10^{-6}
7	2 mL	2.0 mL	0.47	4.7×10^{-6}

Known Addition Technique

Known addition is a convenient technique for measuring samples in the linear range of the electrode (greater than 0.4 ppm or 10^{-5} M calcium) because no calibration curve is required. It can be used to verify the results of a direct calibration or to measure the total concentration of an ion in the presence of a large excess of a complexing agent. The sample potential is measured before and after addition of a standard solution.

Accurate results require that the following conditions be met:

- Concentration should approximately double as a result of the addition.
- Sample concentration should be known to within a factor of three.
- Either no complexing agent or a large excess of the complexing agent may be present.
- The ratio of the uncomplexed ion to complexed ion must not be changed by addition of the standard.
- All samples and standards should be at the same temperature.
- With double or multiple known addition, the final addition should be 10 to 100 times the sample concentration.
- Add 2 mL of ISA to every 100 mL of sample before analysis.

Known Addition Setup

1. Prepare the electrode as described in the **Electrode Preparation** section.

2. Connect the electrode to the meter.

3. Prepare a standard solution that will cause the calcium concentration of the sample to double when added to the sample solution. Refer to **Table 3** for guidelines.

4. Determine the electrode slope by performing the procedure in the **Checking Electrode Operation (Slope)** section.

5. Rinse the electrode with distilled water.

Table 3
Guideline For Known Addition

Volume of Addition	Concentration of Standard
1 mL	100 times sample concentration
5 mL	20 times sample concentration
10 mL*	10 times sample concentration

* Most convenient volume to use

Known Addition Using a Meter with a Known Addition Mode

Note: *See the meter user guide for more specific information.*

1. Set the meter to measure in the known addition mode.

2. Measure 100 mL of the sample and 2 mL of ISA and pour the solutions into a beaker. Rinse the electrode with distilled water and place it into the sample solution. Stir the solution thoroughly.

3. When a stable reading is displayed, set the meter as described in the meter user guide, if required.

4. Pipet the appropriate amount of the standard solution into the beaker. Stir the solution thoroughly.

5. When a stable reading is displayed, record the sample concentration.

Known Addition Using a Meter with a Millivolt Mode

1. Set the meter to the relative millivolt mode. If a relative millivolt mode is not available, use the millivolt mode.

2. Measure 100 mL of sample and 2 mL of ISA and pour the solutions into a 150 mL beaker. Stir the solution thoroughly.

3. Rinse the electrode with distilled water, blot it dry and place the electrode into the beaker. When a stable reading is displayed, set the meter to read 0.0 mV. If the reading cannot be adjusted to 0.0 mV, record the actual mV value.

4. Pipet the appropriate amount of standard solution into the beaker. Stir the solution thoroughly.

5. When a stable reading is displayed, record the mV value. If the meter could not be set to 0.0 mV in step 3, subtract the first reading from the second reading to calculate ΔE.

6. Use **Table 5** to find the Q value that corresponds to the change in potential, ΔE. To determine the original sample concentration, multiply Q by the concentration of the added standard:

   $$\mathbf{C_{sample} = Q * C_{standard}}$$

   $C_{standard}$ = standard concentration
   C_{sample} = sample concentration
   Q = value from **Table 5**

   The table of Q values is calculated for a 10% volume change. The equation for the calculation of Q for different slopes and volume changes is given below.

   $$\mathbf{Q = (p * r) / \{[(1 + p) * 10^{\Delta E/S}] - 1\}}$$

   Q = value from **Table 5**
   $\Delta E = E_2 - E_1$
   S = slope of the electrode
   p = volume of standard / volume of sample and ISA
   r = volume of sample and ISA / volume of sample

Calculating Known Addition for Samples using Lotus, Excel, or Quattro Spreadsheets

If it is more convenient, a simple spreadsheet can be set up to calculate the known addition results, using any ratio of sample to addition. A typical worksheet is shown in **Table 4**. The numbers shown are examples, but the formulas and their locations should be copied exactly.

Table 4
Known Addition Calculations using Lotus, Excel, or Quattro Spreadsheets

A	B	C
1		Enter Value
2	Volume of sample and ISA (mL)	102
3	Volume of addition (mL)	10
4	Concentration of addition	10
5	Volume of sample	100
6	Initial mV reading	45.3
7	Final mV reading	63.7
8	Electrode slope	28.2
9		
10		Derived Values
11	Delta E	+C7 - C6
12	Solution volume ratio	+C3/C2
13	Antilog term	+10^ (C11/C8)
14	Sample volume ratio	+C2/C5
15	Q term	+C12*C14/ (((1+C12)*C13)-1)
16	Calculated initial concentration in same units as addition	+C15*C4

Note: *For Excel, use = instead of + at start of formulas.*

Table 5
Q Values for a 10% volume change,
slopes (in column heading) are in units of mV/decade

ΔE	Q Concentration Ratio			
	28.6	**29.1**	**29.6**	**30.1**
2.5	0.2917	0.2957	0.2996	0.3035
2.6	0.2827	0.2867	0.2906	0.2944
2.7	0.2742	0.2781	0.2820	0.2858
2.8	0.2662	0.2700	0.2738	0.2775
2.9	0.2585	0.2623	0.2660	0.2697
3.0	0.2512	0.2550	0.2586	0.2623
3.1	0.2443	0.2480	0.2516	0.2552
3.2	0.2377	0.2413	0.2449	0.2484
3.3	0.2314	0.2349	0.2384	0.2419
3.4	0.2253	0.2288	0.2323	0.2357
3.5	0.2196	0.2230	0.2264	0.2298
3.6	0.2140	0.2174	0.2208	0.2241
3.7	0.2087	0.2121	0.2154	0.2187
3.8	0.2037	0.2070	0.2102	0.2135
3.9	0.1988	0.2020	0.2052	0.2084
4.0	0.1941	0.1973	0.2005	0.2036
4.1	0.1896	0.1927	0.1959	0.1990
4.2	0.1852	0.1884	0.1914	0.1945
4.3	0.1811	0.1841	0.1872	0.1902
4.4	0.1770	0.1801	0.1831	0.1861
4.5	0.1732	0.1762	0.1791	0.1821
4.6	0.1694	0.1724	0.1753	0.1782
4.7	0.1658	0.1687	0.1716	0.1745
4.8	0.1623	0.1652	0.1680	0.1709
4.9	0.1590	0.1618	0.1646	0.1674
5.0	0.1557	0.1585	0.1613	0.1640
5.1	0.1525	0.1553	0.1580	0.1608
5.2	0.1495	0.1522	0.1549	0.1576
5.3	0.1465	0.1492	0.1519	0.1546
5.4	0.1437	0.1463	0.1490	0.1516
5.5	0.1409	0.1435	0.1461	0.1487
5.6	0.1382	0.1408	0.1434	0.1459
5.7	0.1356	0.1382	0.1407	0.1432
5.8	0.1331	0.1356	0.1381	0.1406
5.9	0.1306	0.1331	0.1356	0.1381
6.0	0.1282	0.1307	0.1331	0.1356
6.1	0.1259	0.1283	0.1308	0.1332
6.2	0.1236	0.1260	0.1284	0.1308
6.3	0.1214	0.1238	0.1262	0.1285
6.4	0.1193	0.1217	0.1240	0.1263
6.5	0.1172	0.1195	0.1219	0.1242
6.6	0.1152	0.1175	0.1198	0.1221
6.7	0.1132	0.1155	0.1178	0.1200
6.8	0.1113	0.1136	0.1158	0.1180
6.9	0.1094	0.1117	0.1139	0.1161

ΔE	Q Concentration Ratio			
	28.6	29.1	29.6	30.1
7.0	0.1076	0.1098	0.1120	0.1142
7.1	0.1058	0.1080	0.1102	0.1123
7.2	0.1041	0.1063	0.1084	0.1105
7.3	0.1024	0.1045	0.1067	0.1088
7.4	0.1008	0.1029	0.1050	0.1071
7.5	0.0992	0.1012	0.1033	0.1054
7.8	0.0946	0.0966	0.0986	0.1006
8.0	0.0917	0.0936	0.0956	0.0976
8.3	0.0876	0.0895	0.0914	0.0933
8.5	0.0850	0.0869	0.0887	0.0906
8.8	0.0813	0.0831	0.0849	0.0868
9.0	0.0790	0.0808	0.0825	0.0843
9.3	0.0757	0.0774	0.0791	0.0809
9.5	0.0736	0.0753	0.0770	0.0787
9.8	0.0706	0.0722	0.0739	0.0755
10.0	0.0687	0.0703	0.0719	0.0735
10.3	0.0660	0.0675	0.0691	0.0707
10.5	0.0642	0.0658	0.0673	0.0689
10.8	0.0617	0.0633	0.0648	0.0663
11.0	0.0602	0.0617	0.0631	0.0646
11.3	0.0579	0.0593	0.0608	0.0623
11.5	0.0564	0.0579	0.0593	0.0607
11.8	0.0544	0.0558	0.0572	0.0585
12.0	0.0530	0.0544	0.0558	0.0572
12.3	0.0511	0.0525	0.0538	0.0551
12.5	0.0499	0.0512	0.0525	0.0539
12.8	0.0481	0.0494	0.0507	0.0520
13.0	0.0470	0.0483	0.0495	0.0508
13.3	0.0454	0.0466	0.0478	0.0491
13.5	0.0443	0.0455	0.0468	0.0480
13.8	0.0428	0.0440	0.0452	0.0464
14.0	0.0419	0.0430	0.0442	0.0454
14.3	0.0404	0.0416	0.0427	0.0439
14.5	0.0395	0.0407	0.0418	0.0429
14.8	0.0382	0.0393	0.0404	0.0416
15.0	0.0374	0.0385	0.0396	0.0407
15.5	0.0354	0.0365	0.0375	0.0386
16.0	0.0335	0.0345	0.0356	0.0366
16.5	0.0318	0.0328	0.0337	0.0347
17.0	0.0302	0.0311	0.0320	0.0330
17.5	0.0286	0.0295	0.0305	0.0314
18.0	0.0272	0.0281	0.0290	0.0298
18.5	0.0258	0.0267	0.0275	0.0284
19.0	0.0246	0.0254	0.0262	0.0270
19.5	0.0234	0.0242	0.0250	0.0258
20.0	0.0223	0.0230	0.0238	0.0246
20.5	0.0212	0.0219	0.0227	0.0234
21.0	0.0202	0.0209	0.0216	0.0224

ΔE	Q Concentration Ratio			
	28.6	**29.1**	**29.6**	**30.1**
21.5	0.0192	0.0199	0.0206	0.0213
22.0	0.0183	0.0190	0.0197	0.0204
22.5	0.0175	0.0181	0.0188	0.0195
23.0	0.0167	0.0173	0.0179	0.0186
23.5	0.0159	0.0165	0.0171	0.0178
24.0	0.0152	0.0158	0.0164	0.0170
24.5	0.0145	0.0151	0.0157	0.0162
25.0	0.0139	0.0144	0.0150	0.0155
25.5	0.0132	0.0138	0.0143	0.0149
26.0	0.0126	0.0132	0.0137	0.0142
26.5	0.0121	0.0126	0.0131	0.0136
27.0	0.0116	0.0120	0.0125	0.0131
27.5	0.0110	0.0115	0.0120	0.0125
28.0	0.0106	0.0110	0.0115	0.0120
28.5	0.0101	0.0106	0.0110	0.0115
29.0	0.0097	0.0101	0.0105	0.0110
29.5	0.0093	0.0097	0.0101	0.0105
30.5	0.0085	0.0089	0.0093	0.0097
31.5	0.0078	0.0081	0.0085	0.0089
32.0	0.0074	0.0078	0.0082	0.0085
32.5	0.0071	0.0075	0.0078	0.0082
33.0	0.0068	0.0072	0.0075	0.0079
33.5	0.0065	0.0069	0.0072	0.0076
34.0	0.0063	0.0066	0.0069	0.0072
34.5	0.0060	0.0063	0.0066	0.0070
35.0	0.0058	0.0061	0.0064	0.0067
35.5	0.0055	0.0058	0.0061	0.0064
36.0	0.0053	0.0056	0.0059	0.0062
36.5	0.0051	0.0053	0.0056	0.0059
37.0	0.0049	0.0051	0.0054	0.0057
37.5	0.0047	0.0049	0.0052	0.0055
38.0	0.0045	0.0047	0.0050	0.0052
38.5	0.0043	0.0045	0.0048	0.0050
39.0	0.0041	0.0043	0.0046	0.0048
39.5	0.0039	0.0042	0.0044	0.0046
40.0	0.0038	0.0040	0.0042	0.0045
40.5	0.0036	0.0038	0.0041	0.0043
41.0	0.0035	0.0037	0.0039	0.0041
41.5	0.0033	0.0035	0.0037	0.0040
42.0	0.0032	0.0034	0.0036	0.0038
42.5	0.0031	0.0033	0.0035	0.0037
43.0	0.0029	0.0031	0.0033	0.0035
43.5	0.0028	0.0030	0.0032	0.0034
44.0	0.0027	0.0029	0.0031	0.0032
44.5	0.0026	0.0028	0.0029	0.0031
45.0	0.0025	0.0027	0.0028	0.0030
45.5	0.0024	0.0026	0.0027	0.0029
46.0	0.0023	0.0024	0.0026	0.0028

Calcium Titration Technique

The calcium electrode makes a highly sensitive endpoint detector for titration with EDTA of calcium samples. Titrations are more time consuming than direct electrode measurement, but results are more accurate and reproducible. With careful technique, titrations accurate to ± 0.1% of the total calcium ion concentration of the sample can be performed.

EDTA complexes other cations besides calcium ion. Interferences from other ions, whose EDTA complexes are stable only at certain pH, can be eliminated by performing the titration for calcium ion at a pH of about 10, but not higher than 11 (adjusted with ammonia). In many cases, other interferences can be eliminated by a suitable choice of sample pH and the addition of masking agents to the sample solution. A comprehensive list of methods is given in the Handbook of Analytical Chemistry, L. Meites, (ed.) McGraw Hill Book Co., New York, (1st edit.), pp. 3-76, 3-225.

Calcium Titration Setup

1. Prepare the electrode as described in the **Electrode Preparation** section. If using the 9720BNWP combination calcium electrode, fill the electrode with Cat. No. 900061. If using the 9320BN half-cell calcium electrode with the 900100 reference electrode, fill the reference electrode with 4 M KCl saturated with Ag/AgCl filling solution, Cat. No. 900011.

2. Connect the electrode to the meter.

3. Prepare a 1 M EDTA stock solution by adding 38.0 grams of reagent-grade Na_4EDTA to a 100 mL volumetric flask. Dissolve the solids with about 75 mL of distilled water and then dilute to the mark with distilled water.

4. Prepare an EDTA titrant solution 10 to 20 times as concentrated as the sample by dilution of the 1 M EDTA stock solution. For a good endpoint break, the sample concentration should be at least 10^{-3} M in total calcium.

Calcium Titration Procedure

1. Place 100 mL of sample into a 150 mL beaker and adjust the pH of the sample to 10 using ammonia. Place the electrode in the sample and stir the solution thoroughly.

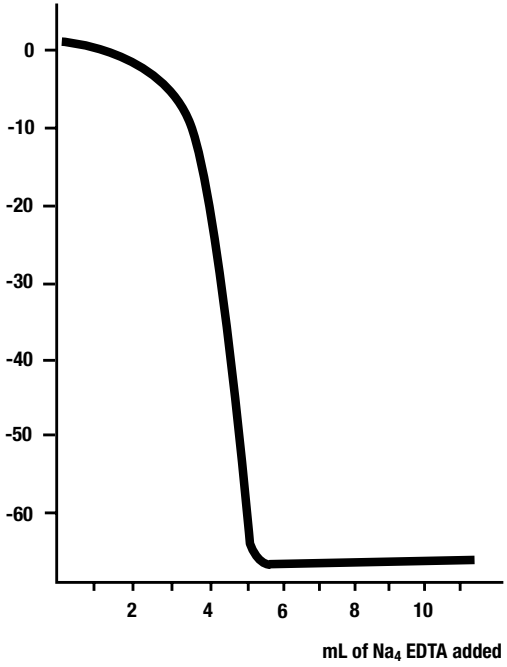
2. Using a 10 mL burette, add increments of the EDTA titrant and plot the electrode potential against mL of titrant added. The endpoint is the point of greatest slope (inflection point). See **Figure 4**.

3. Calculate the sample concentration before dilution:

   $$\mathbf{C_{sample} = C_t\ (V_t\ /\ V_{sample})}$$

   C_{sample} = sample concentration
   C_t = titrant concentration
   V_{sample} = sample volume
   V_t = titrant volume added at endpoint.

Figure 4
Typical Titration of 100 mL of 5 x 10^{-3} M $CaCl_2$ with 0.1 M Na_4EDTA when pH was adjusted to 10 with ammonia



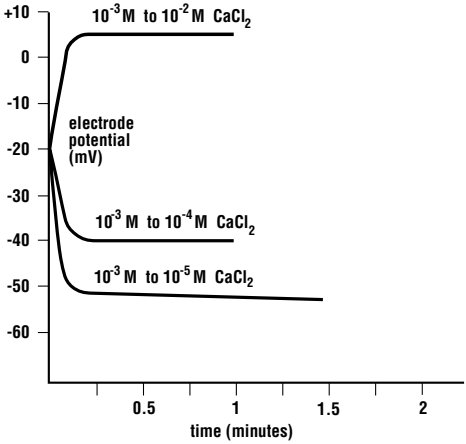
Electrode Characteristics

Electrode Response

The electrode potential plotted against concentration on semi-logarithmic paper results in a straight line with a slope of about 25 to 30 mV per decade change in concentration.

The time response of the electrode (the time required to reach 99% of the stable potential reading) varies from several seconds in concentrated solutions to several minutes near the limit of detection.

Figure 5
Typical Electrode Response to $CaCl_2$ Concentration



Reproducibility

Reproducibility is limited by factors such as temperature fluctuations, drift and noise. Within the operating range of the electrode, reproducibility is independent of concentration. With hourly calibrations, direct electrode measurements reproducible to ± 4 % can be obtained.

Limits of Detection

In pure calcium solutions, the upper limit of detection is 1 M. When possible, dilute the sample into the linear range of the electrode. If samples are not diluted, the possibility of a liquid reference junction potential and the salt extraction effect, need to be considered. At high salt concentrations, salts may be extracted into the electrode membrane, causing deviation from theoretical response. To measure samples between 10^{-1} and 1 M, calibrate the electrode at 4 or 5 intermediate points or dilute the sample.

The lower limit of detection is determined by the slight water solubility of the ion exchanger, which causes deviation from theoretical response. **Figure 3** shows the theoretical response at low levels of calcium compared to the actual response. If calcium measurements are made below 10^{-5} M or 0.4 ppm as Ca^{+2}, a low level measurement procedure is recommended.

Electrode Life

Each sensing module will last approximately six months with normal laboratory use, but the actual lifespan of the sensing module will depend on the type of samples that the electrode is used in. Refer to the **Electrode Maintenance** section for instructions on changing the sensing module. In time, the electrode slope will decrease and readings will start to drift, indicating that the module should be changed. Before replacement, refer to the **Troubleshooting** section to make sure that the difficulties are caused by the sensing module.

Temperature Effects

Since electrode potentials are affected by changes in temperature, samples and standard solutions should be within ± 1 °C (± 2 °F) of each other. At the 10^{-3} M level, a 1 °C difference in temperature results in errors greater than 4 % when using the 9320BN calcium half-cell electrode and errors greater than 1.2 % when using the 9720BNWP calcium combination electrode. The absolute potential of the reference electrode changes slowly with temperature because of the solubility equilibria on which the electrode depends. The slope of the electrode also varies with temperature, as indicated by the factor S in the Nernst equation. Theoretical values of the slope at different temperatures are given in **Table 6**. If the temperature changes, the meter and electrode should be recalibrated.

The electrode can be used at temperatures from 0 to 40 °C, provided that temperature equilibrium has occurred. For use at temperatures substantially different from room temperature, calibration standards should be at the same temperature as samples.

Table 6
Theoretical Slope vs. Temperature Values

Temperature (°C)	Slope (mV)
0	27.1
10	28.1
20	29.1
25	29.6
30	30.1
40	31.1

If sample temperatures vary, use of the 9720BNWP combination calcium electrode is recommended. The Optimum Results A filling solution that is included with the electrode will minimize junction potentials and provide optimum temperature and time response. Optimum Results A filling solution produces an isopotential point of 2.7×10^{-2} M calcium. The isopotential point is the concentration at which the potential of the electrode does not vary with temperature. Since the isopotential point of this electrode is known, the combination calcium electrode may be used on meters that allow automatic temperature compensation for ISE measurements. By programming in the isopotential point and placing an ATC probe into the sample, any time the temperature changes the meter will automatically adjust the slope of the calibration curve, resulting in more accurate measurement results.

Complexation and Precipitation

The electrode responds to the free calcium ions in solution, and not to calcium which is bound or complexed. Certain calcium compounds are relatively insoluble. Among them, in the order of least soluble first, are calcium oxalate, carbonate, fluoride, phosphate and sulfate. For example, in a 40 ppm calcium solution, as little as 10 ppm of fluoride would cause part of the calcium to precipitate as calcium fluoride. About 650 ppm of sulfate would cause the precipitation of calcium at concentrations above about 400 ppm. These solubilities are affected by pH, with the solubility increasing in more acidic solutions. Calcium carbonate precipitation can be avoided completely by operating at pH levels less than 7 and with the total carbonate and bicarbonate concentration kept below 3×10^{-3} M (280 ppm).

Calcium forms soluble complexes with certain inorganic species, such as hydroxide, bicarbonate and polyphosphates. It also forms complexes with some organic anions, such as citrate, tartrate, and EDTA. The extent of complexation increases with increasing calcium concentration and more alkaline pH, and will vary with the sample ionic strength. When it is desired to measure the total calcium concentration in the presence of complexing agents, this can often be done by adding a large excess of complexing agent, and then measuring by known addition.

Interferences

Cations, if present at high enough levels, are electrode interferences and will cause measurement errors. **Table 7** indicates levels of common cations that will cause 10% errors at different concentrations of calcium.

If the electrode is exposed to high levels of interfering ions, it may drift and become sluggish in response. When this happens, restore normal performance by soaking the electrode for an hour in distilled water, emptying the old filling solution, filling the electrode with fresh filling solution and then soaking the electrode for a few hours a 10^{-2} M or 100 ppm calcium standard. If soaking the electrode does not restore normal electrode performance, refer to the **Electrode Maintenance** section for instructions on how to replace the sensing module.

When the level of interferences in samples is constant, it is sometimes possible to measure calcium accurately when interference levels are higher than those in **Table 7**. For example, calcium can be measured in sea water by using synthetic ocean water for calibration. Contact our Technical Support Chemists for more information.

Table 7
Calcium Electrode Interferences

Interferences Moles/Liter	10^{-4} M Calcium	10^{-3} M Calcium	10^{-2} M Calcium
Pb^{+2}	1.0×10^{-6}	1.0×10^{-5}	1.0×10^{-4}
Hg^{+2}	4.0×10^{-4}	4.0×10^{-3}	4.0×10^{-2}
H^{+}	4.0×10^{-4}	4.0×10^{-3}	4.0×10^{-2}
Sr^{+2}	6.0×10^{-4}	6.0×10^{-3}	6.0×10^{-2}
Fe^{+2}	2.0×10^{-3}	2.0×10^{-2}	0.2
Cu^{+2}	4.0×10^{-3}	4.0×10^{-2}	0.4
Ni^{+2}	5.0×10^{-3}	5.0×10^{-2}	0.5
NH_4^{+}	2.0×10^{-2}	0.2	2.0
Na^{+}	2.0×10^{-2}	0.2	2.0
$Tris^{+}$ *	3.0×10^{-2}	0.3	3.0
Li^{+}	3.0×10^{-2}	0.3	3.0
K^{+}	4.0×10^{-2}	0.4	4.0
Ba^{+2}	7.0×10^{-2}	0.7	7.0
Zn^{+2}	0.1	1.0	10
Mg^{+2}	0.1	1.0	10

Interferences ppm	1 ppm Calcium as $CaCO_3$	10 ppm Calcium as $CaCO_3$	100 ppm Calcium as $CaCO_3$
Pb^{+2}	0.2	2	20
Hg^{+2}	80	800	8000
H^{+}	3.4 pH	2.4 pH	1.4 pH
Sr^{+2}	52	520	5200
Fe^{+2}	111	1110	11100
Cu^{+2}	254	2540	25400
Ni^{+2}	294	2940	29400
NH_4^{+}	340	3400	34000
Na^{+}	460	4600	46000
$Tris^{+}$ *	3630	36300	363000
Li^{+}	208	2080	20800
K^{+}	1564	15640	156400
Ba^{+2}	9614	96140	961400
Zn^{+2}	6537	65370	653700
Mg^{+2}	2430	24300	243000

* Tris is the cation of tris(hydroxymethyl) aminomethane.

pH Effects

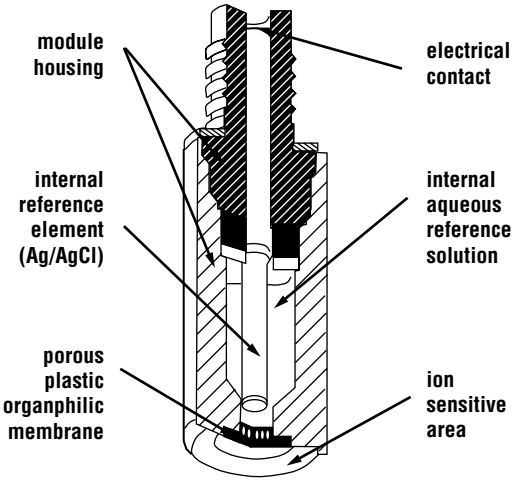
Although the electrode can be used over a wide pH range, hydrogen ion interferes with measurements of low levels of calcium ion. Refer to **Table 7** to determine the minimum pH at which low level calcium measurements can be made without more than a 10% error due to hydrogen ion interference.

At high pH, sufficient hydroxide ion is present to form a precipitate with a portion of the calcium ion. This reaction reduces the level of free calcium ion in the sample. Since the electrode responds only to free, unbound calcium ion, it does not detect that portion of the sample calcium precipitated by hydroxide ion. Precipitation can be avoided by adjusting pH of sample and standards to below pH 11 with 1 M HCl when necessary.

Theory of Operation

The calcium electrode consists of a replaceable, pretested sensing module connected to an epoxy body. The sensing module contains a liquid internal filling solution in contact with a gelled organophilic membrane that contains a calcium selective ion exchanger.

Figure 6
Example of an Ion Sensing Module



When the module is in contact with a solution containing calcium ions, an electrode potential develops across the module. This potential, which depends on the level of free calcium ion in solution, is measured against a constant reference potential with a digital pH/mV meter or ISE (concentration) meter. The measured potential corresponding to the level of calcium ion in solution is described by the Nernst equation.

$$E = E_o + S * \log (A)$$

E = measured electrode potential
E_o = reference potential (a constant)
A = calcium ion activity level in solution
S = electrode slope (about 28 mV per decade)
S = (2.3 R T) / nF
R and F are constants, T = temperature in degrees K and n = ionic charge

The level of calcium ions, A, is the activity or "effective concentration" of free calcium ions in solution. The calcium ion activity is related to free calcium ion concentration, C_f, by the activity coefficient, y.

$$A = y * C_f$$

Ionic activity coefficients are variable and largely depend on total ionic strength. The ionic strength of a solution is determined by all of the ions present. It is calculated by multiplying the concentration of each individual ion by the square of its charge, adding all these values up and then dividing by two.

$$\textbf{Ionic strength} = 1/2 \sum (C_i Z_i^2)$$

C_i = concentration of ion i
Z_i = charge of ion i
$\sum$ symbolizes the sum of all the types of ions in solutions

If background ionic strength is high and constant relative to the sensed ion concentration, the activity coefficient is constant and activity is directly proportional to concentration. Ionic strength adjustor (ISA) is added to all calcium standards and samples so that the background ionic strength is high and constant relative to variable concentrations of calcium. For calcium, the recommended ISA is NaCl. Other solutions can be used as long as they do not contain ions that would interfere with the electrode response to calcium.

If samples have a high ionic strength (above 0.1 M), standards should be prepared with a composition similar to the samples.

Reference electrode conditions must also be considered. Liquid junction potentials arise any time when two solutions of different composition are brought into contact. The potential results from the interdiffusion of ions in the two solutions. Since ions diffuse at different rates, the electrode charge will be carried unequally across the solution boundary resulting in a potential difference between the two solutions. In making electrode measurements, it is important that this potential is the same when the reference is in the standardizing solution as well as in the same solution; otherwise, the change in liquid junction potential will appear as an error in the measured specific ion electrode potential.

The most important variable that analysts have under their control is the composition of the liquid junction filling solution. The filling solution should be equitransferent. That is, the speed with which the positive and negative ions in the filling solution diffuse into the sample should be nearly as equal as possible. If the rate at which positive and negative charge is carried into the sample solution is equal, then no junction potential can result. Optimum Results filling solutions are specifically designed to meet all reference electrode conditions.

Troubleshooting

Follow a systematic procedure to isolate the problem. The measuring system can be divided into four components for ease in troubleshooting: meter, electrode, sample/application and technique.

Meter

The meter is the easiest component to eliminate as a possible cause of error. Thermo Scientific Orion meters include an instrument checkout procedure and shorting cap for convenience in troubleshooting. Consult the meter user guide for directions.

Electrode

1. Rinse the electrode thoroughly with distilled water.

2. Verify the electrode performance by performing the procedure in the **Checking Electrode Operation (Slope)** section.

3. If the electrode fails this procedure, review the **Measuring Hints** section. Clean the electrode thoroughly as directed in the **Electrode Maintenance** section. Drain and refill the electrode with fresh filling solution.

4. Repeat the procedure in the **Checking Electrode Operation (Slope)** section.

5. It the electrode fails this procedure again and the half-cell calcium electrode is being used, determine whether the calcium or reference electrode is at fault. To do this, substitute a known working electrode for the electrode in question and repeat the procedure in the **Checking Electrode Operation (Slope)** section.

6. If the electrode passes the procedure, but measurement problems persist, the sample may contain interferences or complexing agents, or the technique may be in error.

7. Before replacing a faulty electrode, review this user guide and be sure to thoroughly clean the electrode; correctly prepare the electrode; use the proper filling solution, ISA, and standards; correctly measure the samples and review the **Troubleshooting Checklist** section.

Sample/Application

The quality of results depends greatly upon the quality of the standards. Always prepare fresh standards when problems arise, it could save hours of frustrating troubleshooting! Errors may result from contamination of prepared standards, accuracy of dilution, quality of distilled water, or a mathematical error in calculating the concentrations.

The best method for preparation of standards is serial dilution. Refer to the **Serial Dilution** section. The electrode and meter may operate with standards, but not with the sample. In this case, check the sample composition for interferences, incompatibilities or temperature effects. Refer to the **Sample Requirements**, **Temperature Effects**, **Interferences, Complexation and Precipitation** and **pH Effects** sections.

Technique

If trouble persists, review operating procedures. Review calibration and measurement sections to be sure proper technique has been followed. Verify that the expected concentration of the ion of interest is within the limit of detection of the electrode.

Check the method of analysis for compatibility with your sample. Direct measurement may not always be the method of choice. If a large amount of complexing agents are present, known addition may be the best method. If working with low level samples, follow the procedure in the **Low Level Calibration** section.

Assistance

After troubleshooting all components of your measurement system, contact Technical Support. Within the United States call 1.800.225.1480 and outside the United States call 978.232.6000 or fax 978.232.6031. In Europe, the Middle East and Africa, contact your local authorized dealer. For the most current contact information, visit www.thermo.com/contactwater.

For the latest application and technical resources for Thermo Scientific Orion products, visit www.thermo.com/waterapps.

Warranty

For the most current warranty information, visit www.thermo.com/water.

Troubleshooting Checklist

- No electrode filling solution added –
  Fill the electrode with filling solution up to the fill hole. Refer to the **Electrode Preparation** section for details.

- Incorrect electrode filling solution used –
  Refer to the **Electrode Preparation** section to verify that the correct electrode filling solution was used.

- Electrode junction is dry –
  Push down on the electrode cap to allow a few drops of filling solution to drain out of the electrode.

- No reference electrode present –
  The 9320BN calcium half-cell electrode require a separate reference electrode, Cat. No. 900100.

- Electrode is clogged or dirty –
  Refer to the **Electrode Maintenance** section for electrode cleaning and flushing instructions.

- Sensing module is not installed properly, dirty or defective –
  Refer to the **Electrode Preparation** section and verify that the electrode was assembled correctly. Refer to the **Electrode Maintenance** section for instructions on installing a new sensing module.

- Standards are contaminated or made incorrectly –
  Prepare fresh standards. Refer to the **Serial Dilution**, **Measurement Hints** and **Analytical Techniques** sections.

- ISA not used or incorrect ISA used –
  ISA must be added to all standards and samples. Refer to the **Required Equipment** section for information on the ISA.

- Samples and standards at different temperatures –
  Allow solutions to reach the same temperature.

- Air bubble on sensing module –
  Remove air bubble by reimmersing the electrode in solution.

- Electrode not properly connected to meter –
  Unplug and reconnect the electrode to the meter.

- Meter or stir plate not properly grounded –
  Check the meter and stir plate for proper grounding.

- Static electricity present –
  Wipe plastic parts on the meter with a detergent solution.

- Defective meter –
  Check the meter performance. See the meter user guide.

Ordering Information

Cat. No.	Description
9720BNWP	Calcium ionplus Sure-Flow combination electrode, waterproof BNC connector
900061	Optimum Results A electrode filling solution, 5 x 60 mL bottles
9320BN	Calcium half-cell electrode, BNC connector (requires separate reference electrode)
900100	Single junction reference electrode, pin tip connector
900011	4 M KCl saturated with Ag/AgCl filling solution, 5 x 60 mL bottles
922006	0.1 M $CaCl_2$ calcium standard, 475 mL bottle
923206	100 ppm calcium as $CaCO_3$ standard, 475 mL bottle
932011	ISA for calcium measurements, 475 mL bottle
972001	97 series sensing module for 9720BNWP calcium combination electrode
9700BNWP	Replacement body for 9720BNWP calcium combination electrode (requires separate 97 series sensing module)
932001	93 series sensing module for 9320BN calcium half-cell electrode
9300BNWP	Replacement body for 9320BN calcium half-cell electrode (requires separate 93 series sensing module)

Specifications

Concentration Range

5 x 10^{-7} M to 1 M (0.02 ppm to 40,100 ppm as Ca^{+2} or 0.05 to 100,000 ppm as $CaCO_3$)

pH Range

2.5 to 11
Low level measurements may be influenced by hydrogen or hydroxide ion interferences

Temperature Range

0 to 40 °C

Electrode Resistance

0.1 to 4 megohms

Reproducibility

± 4%

Minimum Sample Size (9720BNWP)

5 mL in a 50 mL beaker

Size– 9720BNWP

Body Diameter: 13 mm

Body Length: 110 mm

Cap Diameter: 16 mm

Cable Length: 1 meter

Size– 9320BN

Body Diameter: 12 mm

Body Length: 110 mm

Cap Diameter: 16 mm

Cable Length: 1 meter

** Specifications are subject to change without notice*

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