All about pH!
Is it as simple as it seems?
The pH Workshop

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Thermo Scientific

Water Analysis Instruments, Orion products

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Common Questions: *Measuring pH*

What is pH?

"Potential Hydrogen" or "Power of Hydrogen"

pH electrodes are a type of ion selective electrode (ISE) measuring free hydrogen ion activity

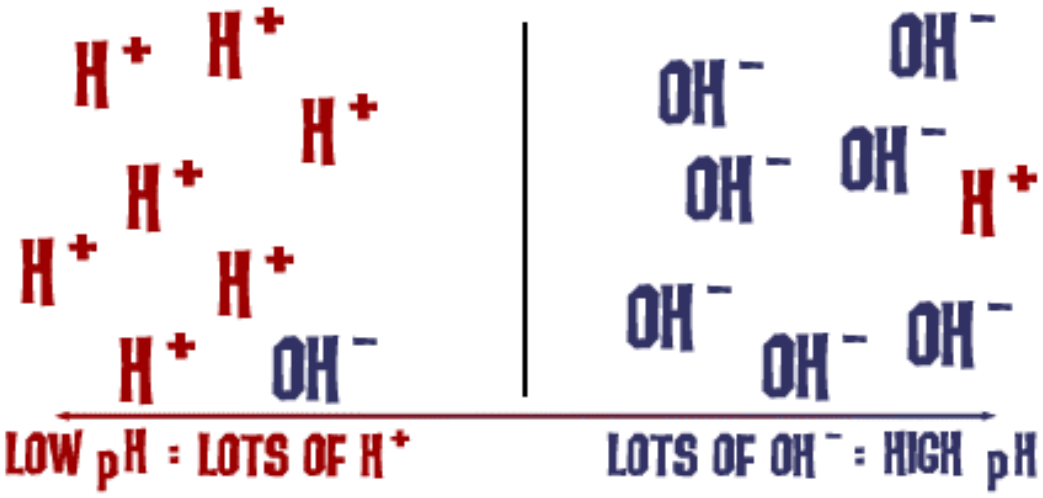
Common Questions: *What is pH?*

The Theoretical Definition: $\mathbf{pH = - \log a_H}$

- a_H is the hydrogen ion *activity*.
- In solutions that contain other ions, activity and concentration are not the same.
- The activity is an *effective* concentration of hydrogen ions, rather than the true concentration; it accounts for the fact that other ions surrounding the hydrogen ions will shield them and affect their ability to participate in chemical reactions.
- These other ions effectively change the hydrogen ion concentration in any process that involves H^+.

What is pH?

- pH = “Potential Hydrogen” or Power of Hydrogen
- The pH of pure water around room temperature is about 7. This is considered "neutral" because the concentration of hydrogen ions (H^+) is exactly equal to the concentration of hydroxide (OH^-) ions produced by dissociation of the water.
- Increasing the concentration of H^+ in relation to OH^- produces a solution with a pH of less than 7, and the solution is considered "acidic".
- Decreasing the concentration H^+ in relation to OH^- produces a solution with a pH above 7, and the solution is considered "alkaline" or "basic".



What is pH?

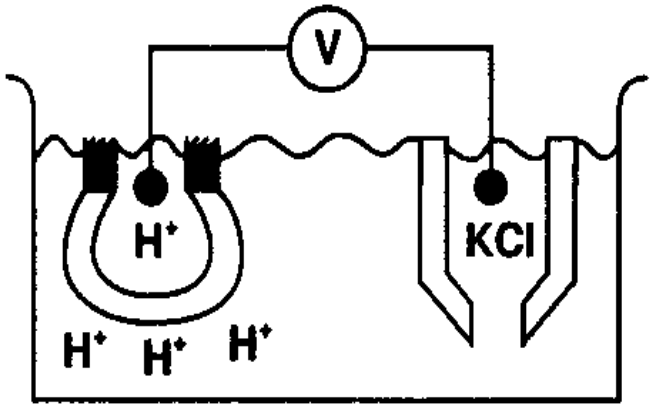
- The pH Scale
- Each pH unit is a factor 10 in $[H^+]$
  - pH of Cola is about 2.5. This is 10x more acidic than Orange Juice (pH of 3.5).
  - Cola is 100x more acidic than Beer!

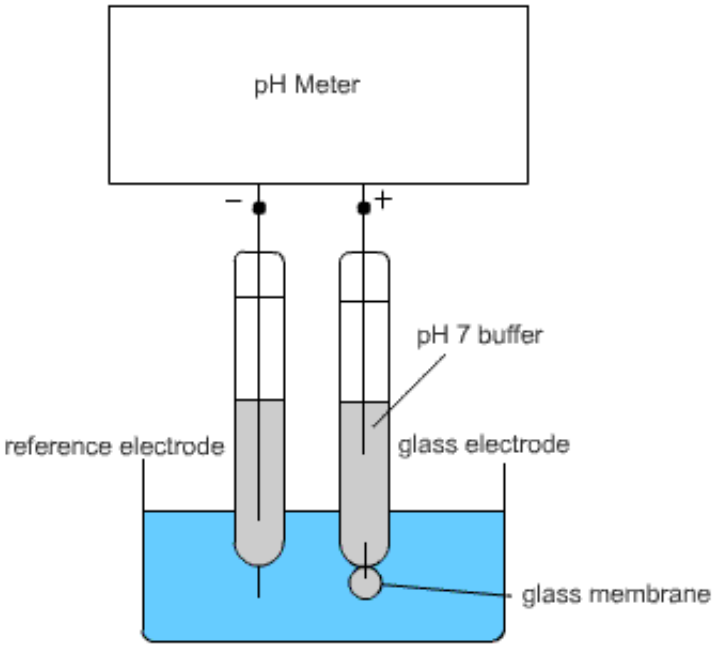
Representative pH values

Substance	pH
Hydrochloric Acid, 10M	-1.0
Lead-acid battery	0.5
Gastric acid	1.5 – 2.0
Lemon juice	2.4
Cola	2.5
Vinegar	2.9
Orange or apple juice	3.5
Beer	4.5
Acid Rain	<5.0
Coffee	5.0
Tea or healthy skin	5.5
Milk	6.5
Pure Water	7.0
Healthy human saliva	6.5 – 7.4
Blood	7.34 – 7.45
Seawater	7.7 – 8.3
Hand soap	9.0 – 10.0
Household ammonia	11.5
Bleach	12.5
Household lye	13.5

pH Measurement System

- When two solutions containing different concentrations of H^+ ions are separated by a glass membrane, a voltage potential is developed across the membrane. (Sensing electrode)
- A voltage potential is also generated from the reference electrode.
- The pH meter measures the voltage potential difference (mV) between the sensing electrode measuring the outside sample and a stable reference electrode and translates this to the pH scale.





pH Measurement System

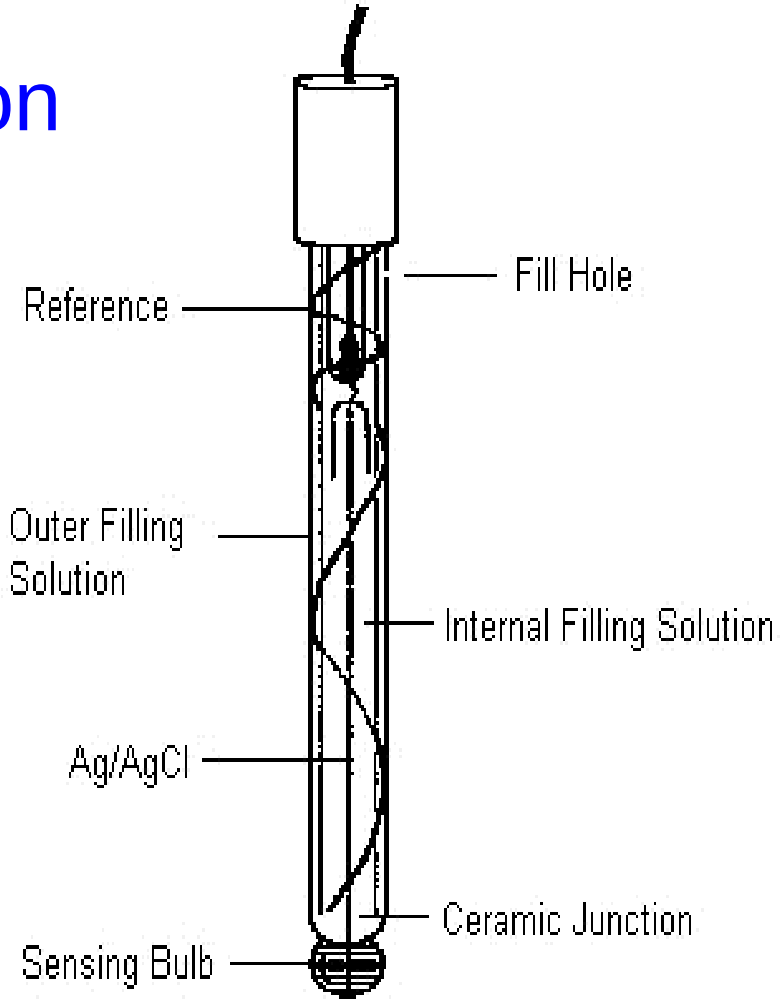
- The pH Meter
  - Acts as a volt meter
  - Translates electrode potential (mV) to pH scale
- Meter functions
  - Stores calibration curve
  - Adjusts for temperature changes
  - Adjusts electrode slope
  - Signals when reading is stable
- Features
  - mV and relative mV scales
  - Autocalibration/autobuffer recognition
  - Number of calibration points
  - Display information
  - RS232 or recorder outputs
  - Datalogging
  - GLP/GMP compliant

Common Questions: *Electrode Composition*

pH Electrode Composition

- Sensing Bulb
- Reference
- Reference Solution
- Internal Fill Solution
- Junction

This is a "combination pH electrode"



pH Electrode Reference Types

- Calomel reference
  - *Fixed Hg_2^{++} activity in contact with solid mercury*
- Silver reference
  - *Fixed Ag+ activity in contact with silver wire*
  - *Single and double junction design*
- ROSS reference
  - *Redox couple (Iodide/Iodine)*
  - *Double junction design*

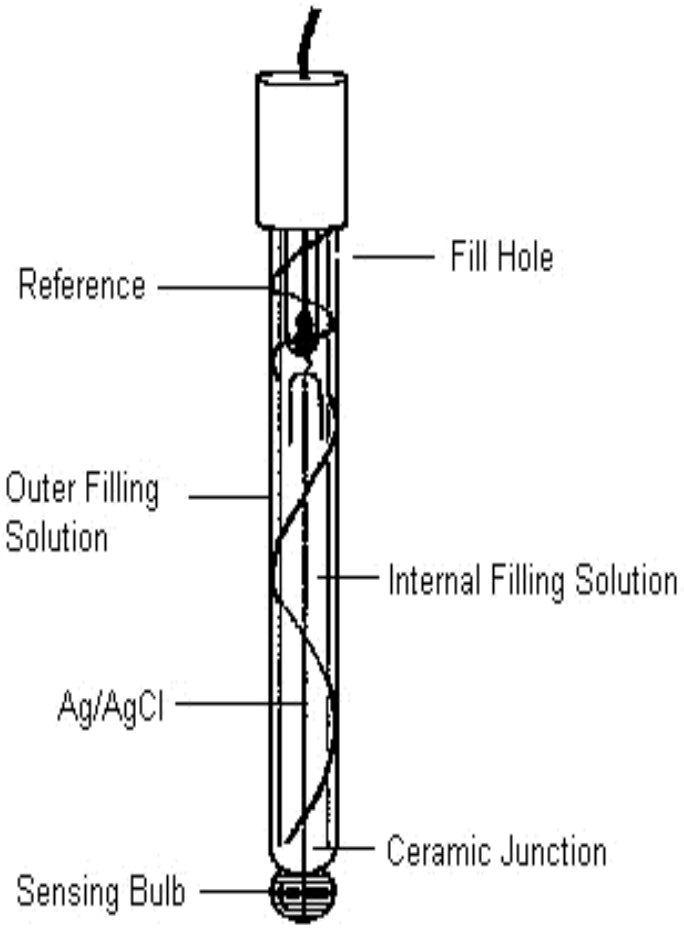
Common Questions: *Electrode Components*

pH Electrode Junction Types

- Wick junction
  - *Glass fiber, fiber optical bundles, Dacron, etc.*
  - *Common in epoxy body electrodes*
- Ceramic junction
  - *Porous ceramic, wooden plug, porous Teflon, etc.*
  - *Common in glass body electrodes*

pH Measurement System - Junctions

- The electrode junction is where the Outer fill solution (reference) passes from inside the electrode body to the sample completing the “circuit”.
- The type of junction is a good indicator of how the electrode will perform in different samples.
- Three basic types of junctions
  - Wick
  - Ceramic
  - Open



pH Measurement System - Junctions

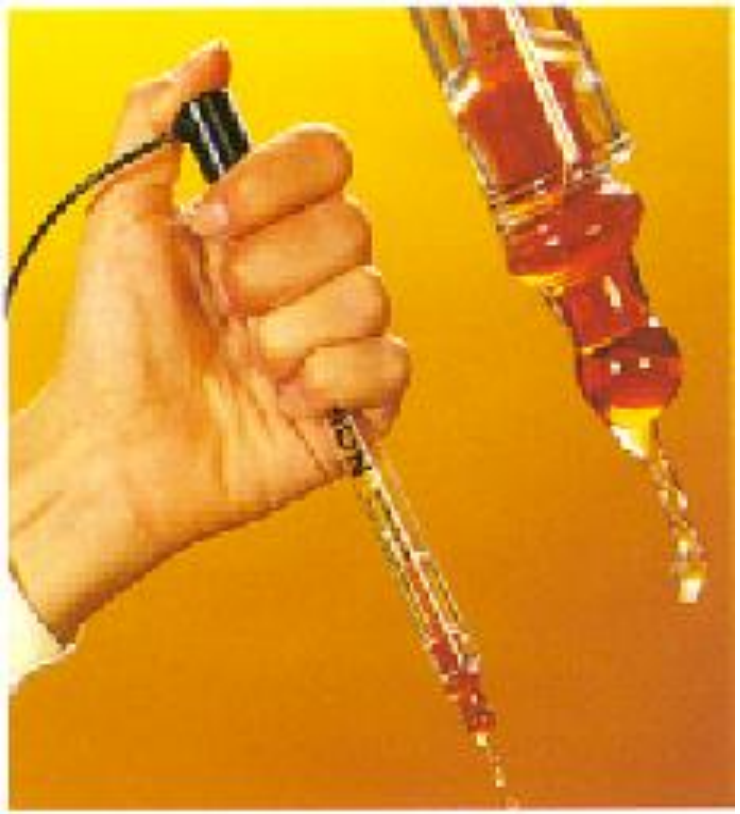
- The Wick Junction
  - Glass fiber, fiber optic bundles, Dacron, etc.
- Advantages
  - Used in rugged epoxy bodies
  - Good for aqueous samples
- Disadvantages
  - Will clog if sample is “dirty” or viscous
  - Not as “fast” as other junctions

pH Measurement System - Junctions

- The Ceramic Junction
  - Porous ceramics, wooden plugs, porous teflon, etc.
- Advantages
  - Good all-purpose junction
  - Ideally suited for most lab applications
- Disadvantages
  - Will clog if sample is “dirty” or viscous

pH Measurement System - Junctions

- The Open Junction
  - Sure-Flow or Ground Glass Sleeve, Laser Drilled Hole,, or Bundle of Capillary Tubes, etc.
- Advantages
  - Sure-Flow or Sleeve Junction will never clog
  - Can be used in all sample types
  - Ideal choice for “dirty” or viscous samples
  - Can be used in non-aqueous samples
- Disadvantages
  - Sure-Flow Junction has a high flow rate of fill solution (2 ml/day)



pH Measurement System – Electrode Types

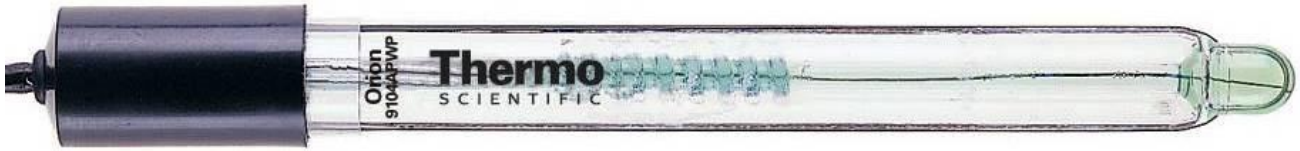
- Refillable or Low Maintenance Gel?
- Low Maintenance Gel Electrodes
  - Easy to use
  - Rugged epoxy body
  - 0.05-0.1 pH precision
  - Slower response rate
  - 6 month average life
  - Gel memory effects at junction
- Refillable Electrodes
  - Fill/drain electrode
  - Wide applicability
  - Glass or epoxy body
  - 0.02 pH precision
  - Faster response rate
  - 1 year minimum life
  - Replaceable fill solution

Common Questions: *Electrode Types*

What is meant by a "single junction?"

- *There is one junction in the electrode body.*

  This term applies to calomel electrodes or Ag/AgCl electrodes that have a silver reference wire and silver ions dispersed in the internal electrolyte fill solution.



Common Questions: *Electrode Types*

What is meant by a "double junction?"

- *There are two junctions in the electrode body.*

  This term applies to any electrode that has a ROSS reference and also to some Ag/AgCl electrodes.

pH Measurement System - Electrode Selection

- Select proper reference for application
  - ROSS™, Single or Double Junction Ag/AgCl
  - Remember that Calomel contains Mercury!
- Select proper junction for application
  - Wick, Ceramic, Open, Sure-Flow, etc.
- Select appropriate body style
  - Standard, semi-micro, micro, rugged bulb, spear tip, flat surface
- Select appropriate body type
  - Glass body, epoxy body
- Other considerations
  - Refillable, Gel, or Polymer?
  - Built in Temperature Probe?

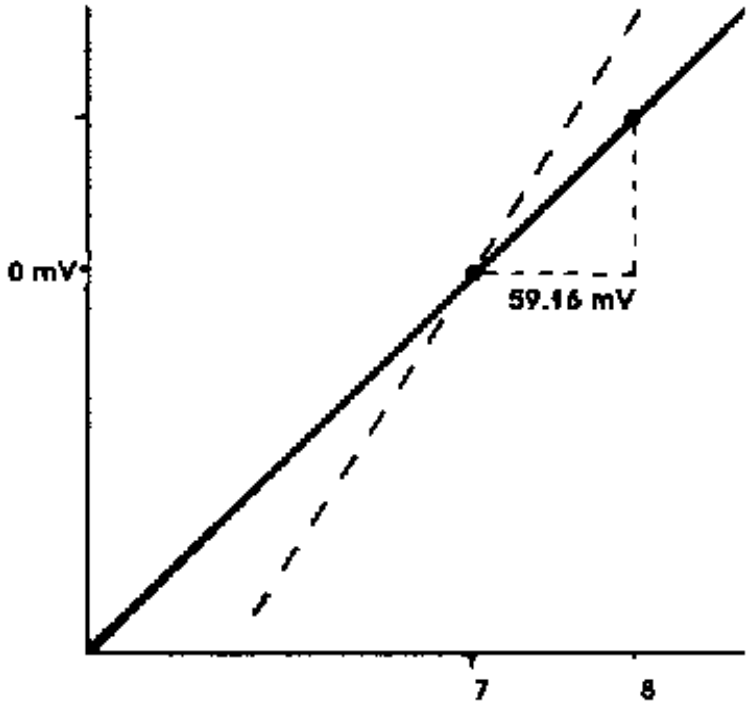
pH Calibration

- The Nernst Equation

$$E = E_0 - RT/nF \log a_H$$

E = measured potential
E_0 = reference potential
R = Universal Gas Constant
T = Temperature (at 25 ^oC)
n = Number of electrons
F = Faraday Constant
a_H = Hydrogen Ion activity

Slope = RT/nF = 59.16mv @ 25 ^oC



pH Calibration

- When you are calibrating, you are determining the electrodes slope as it relates to the theoretical slope defined by the Nernst Equation
- Newer meters automatically calculate slope
- Check slope manually by reading mV in buffers and comparing to Nernstian response (59.2 mV/pH unit)
  - *Example:*
    - pH 7 = -10 mV
    - pH 4 = +150 mV
    - 150 – (-10) = 160 mV
    - 3 pH units x 59.2mV = 177.6 mV
    - Slope = 160 mV/177.6 mV x 100 = 90.1%

pH Calibration - Guidelines

- Always calibrate with at least 2 buffers
- Check calibration drift with 1 buffer
- Always calibrate with buffers that bracket the expected measurement range
- Calibrate with buffers that are no more than 3 pH units apart
- Track calibration slope on a daily basis
- Calibration frequency
  - Electrode type
  - Sample type
  - Number of samples
- Electrode slope guidelines
  - Ideal range: 95% - 102%

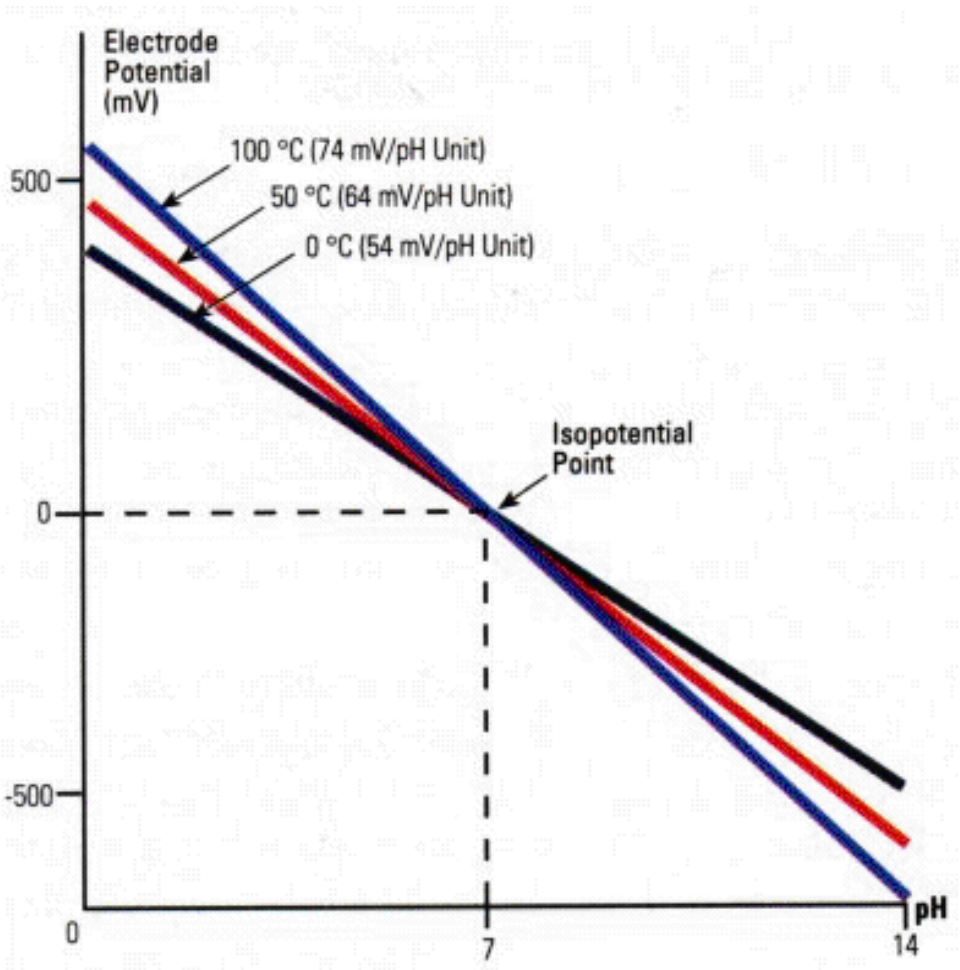
Common Questions: *Temperature Compensation*

Why is temperature compensation important when measuring pH ?

- *Samples / buffers have different pH values at different temperatures*
- *Temperature compensation will contribute to achieving accurate measurements*

Common Questions: *Temperature Compensation*

- Temperature affects calibration slope because it affects the expected change in the mV value per pH unit
- *Temperature compensation will adjust the calibration slope* across a wide temperature range
- *It is not possible to normalize pH readings to a specific temperature,* but it is possible to get an accurate pH measurement for any sample temperature



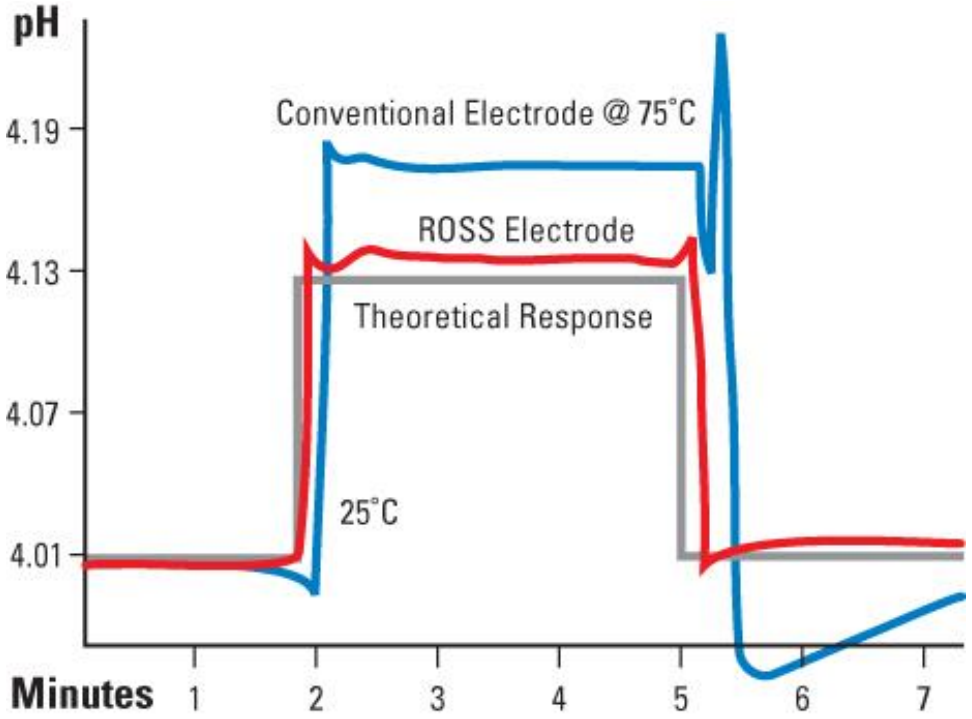
Common Questions: *Temperature Compensation*

Temperature Compensation Strategies

- *Calibrate and measure at the same temperature*
- *Use automatic temperature compensator (ATC) or 3-in-1 Triode electrode*
- *Manually temperature compensate using temperature control on meter*
- *Use LogR temperature compensation*
- *Record temperature with pH readings*

Effects of Temperature – Electrode Effects

- Temperature “Hysteresis”
  - AgCl or Hg_2Cl_2 references drift with temperature changes
  - 0.05 pH unit error with 4 ^oC difference
  - ROSS™ electrodes stabilize within seconds
  - With other Ion Selective Electrodes a 1 degree C temperature change creates up to a 2% error



Effects of Temperature – Buffer Effects

- Buffer Effects
  - Buffers have different pH values at different temperatures
  - Use the value of the buffer at the calibration temperature
  - New meters have NIST calibration tables pre-programmed
  - NIST Certified Values only at 25°C

25 C	0 C	5 C	10 C	20 C	30C	40 C	50 C	60 C	70 C	80 C	90 C
1.68	1.67	1.67	1.67	1.67	1.68	1.69	1.71	1.72	1.74	1.77	1.79
3.78	3.86	3.84	3.82	3.79	3.77	3.75	3.75				
4.01	4.00	4.00	4.00	4.00	4.02	4.03	4.06	4.08	4.13	4.16	4.21
6.86	6.98	6.95	6.92	6.87	6.85	6.84	6.83	6.84	6.85	6.86	6.88
7.00*	7.11	7.08	7.06	7.01	6.98	6.97	6.97				
7.41	7.53	7.50	7.47	7.43	7.40	7.38	7.37				
9.18	9.46	9.40	9.33	9.23	9.14	9.07	9.01	8.96	8.92	8.89	8.85
10.01	10.32	10.25	10.18	10.06	9.97	9.89	9.83				
12.46	13.42	13.21	13.01	12.64	12.30	11.99	11.71				

*Non-NIST Phosphate Buffer

Electrode Care and Maintenance

- Electrode Storage
  - Short-term storage
    - Use electrode storage solution
    - Alternatively, soak in 100 ml pH 7 buffer with 0.5 g KCl
  - Long-term storage
    - Fill electrode, close fill hole, store with storage solution in protective cap
- Cleaning Solutions
  - Soak electrode in solvent that will remove deposits
    - Example: 0.1 M HCl for general cleaning
    - Example: 1% pepsin in HCl for proteins
    - Example: Bleach for disinfecting
    - Example: detergent for grease & oil

Electrode Care and Maintenance

- When do you need to clean your electrode?
  - Check slope range
    - Ideal range: 95% - 102%
    - Cleaning range: 92% - 95%
    - Replacement range: below 92%
  - Check response times in buffers
    - Electrode stability within 30 seconds
  - Check precision of electrode by reading buffers as samples
  - Check for any drift of electrode in pH buffer

Electrode Care and Maintenance

- General electrode bulb cleaning
  - Soak in Cleaning Solution for 30 minutes
  - Replace electrode fill solution
  - Soak in storage solution for at least 2 hours
- Electrode junction cleaning
  - Soak in 0.1M KCl for 15 minutes at 70 ^{o}C
  - Replace electrode fill solution
  - Soak in electrode storage solution for 2 hours
- Check junction by suspending in air for ten minutes
  - Observe KCl crystal formation

Keys to Accuracy

- Always use fresh buffers
  - Check bottle expiration and date opened
    - pH 4 and pH 7 buffers expire within 3 months of being opened
    - pH 10 buffer expires within 1 month of being opened
  - Fresh buffer for each calibration
    - Calibrate only once in buffer… don't re-use buffer
- Replace the fill solution in the electrode every week
  - Fill solution concentration is maintained
  - KCl crystallization is prevented
- Make sure to use the correct fill solution
  - Ross electrodes cannot use silver fill solutions

Keys to Accuracy

- Make sure level of fill solution is high
- Gently stir buffers and samples
- Shake any air bubbles out of the electrode
- Use insulation between stir plate and sample container to minimize heat transfer
- Blot electrodes between samples
- Uncover fill hole during measurement

Troubleshooting pH Problems

- Troubleshooting pH Meters
  - Use meter shorting strap
  - Reading should be 0 mV +/- 0.2 mV
  - Use meter self-test procedure

- Troubleshooting Buffers
  - Use Fresh Buffers for calibration
  - Verify expiration date
  - Stir buffers during calibration

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Troubleshooting pH Problems

- Troubleshooting pH Electrodes
  - Clean bulb, junctions
  - Replace Fill solution
  - Uncover fill hole
  - Check for scratches on sensing bulb
- Troubleshooting Samples
  - Proper sample preparation
  - Stir samples
- Troubleshooting Technique
  - Treat samples and buffers the same
  - Clean and blot electrode between samples

Common Questions: *Calibration*

My samples range from pH 5 to 8. Can I use a 4 and 10 standard for my 2-point calibration?

- *The slope (or efficiency) of any electrode will not be consistent across a range of measurement.*
- *The greater the range between calibration points, the greater the measurement error.*
- *Calibration should include at least 2 buffers, but these buffers should be no more than 3 pH units apart from the next sequenced buffer.*
- *The 4-10 slope created across 6 decades of measurement will provide less accuracy than two point-to-point slopes using 4-7 (3 decades) and 7-10 (3 decades)*

Common Questions: *Calibration*

I have small containers on my bench that are labeled and filled with fresh buffer each week.
We re-use these buffers all week.
Will this practice affect my calibration?

Cal 1, using fresh 7 and 10 buffer:

- *slope between 7-10 = 96.7%*

Cal 2, using fresh 7 and old 10 buffer:*

- *slope between 7-10 = 93.4%*

** set on shelf uncovered for 8 hours*

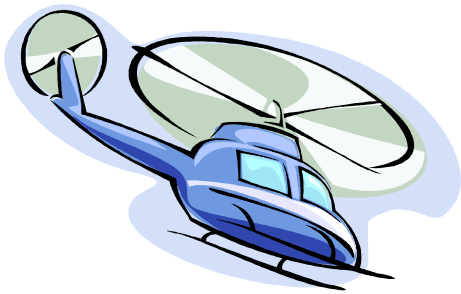
ALWAYS use fresh buffer for each calibration.

Don’t re-use today’s buffer for tomorrow’s calibration!

Common Questions: *Stable Readings*

Why does it take so long to get a stable reading?

- *electrode performance and efficiency*
- *inner fill-solution freshness*
- *electrode type (gel effects, open junction, etc.)*
- *junction and bulb function (non-clogged and non-coated)*
- *meter stabilization settings (if available)*
- *resolution settings*
- *low ionic strength samples*
- *air bubbles near bulb*
- *stirred or not?*



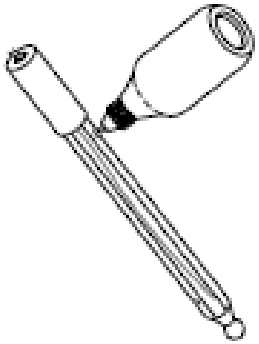
Common Questions: *Stable Readings*

What can be done to improve measurements made in low ionic strength samples?

- *use an electrode with an open junction*
- *stir the samples during measurement*

Common Questions: *Maintenance*

Is there a cleaning routine I can follow to keep my electrode working?



- refresh inner fill solution
- use recommended storage solution
- close fill hole at end of day
- use cleaning remedies if a coated bulb or a clogged junction is the suspected cause of a poor calibration slope

New Technologies

- LogR Temperature Compensation
  - Meter reads the resistance (R) from the bulb of any pH electrode
  - Resistance measurement is inverse to temperature: LogR = 1/T
  - Calibrate pH electrode for temperature
  - Direct temperature compensation without using ATC

New Technologies

- AquaPro Electrodes
  - Low maintenance sealed electrode
    - No fill solutions to worry about
  - Patented polymer reference gel
    - Performance and life equivalent to refillable electrodes
  - Double-junction design
    - Silver will not precipitate with sulfides, TRIS, or proteins
  - Laser-drilled open junction
    - Won’t clog in viscous samples

New Technologies

- **ROSS™ Ultra** Electrodes
  - Best of the Best!
    - *Superior Performance*
    - *Fast Response*
    - *Very Stable*
  - 2-Year Replacement Warranty

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New Technologies

- ROSS™ Micro Electrode
  - Measure samples as small as 15µl in 384 well plates
  - Only needs to be immersed 4.5mm into the sample
  - PerpHect electrode, Ideal for LogR meters!

- Micro ATC probe
  - Stainless Steel
  - Measure samples as small as 10µl
  - Only needs to be immersed 3.0mm into the sample

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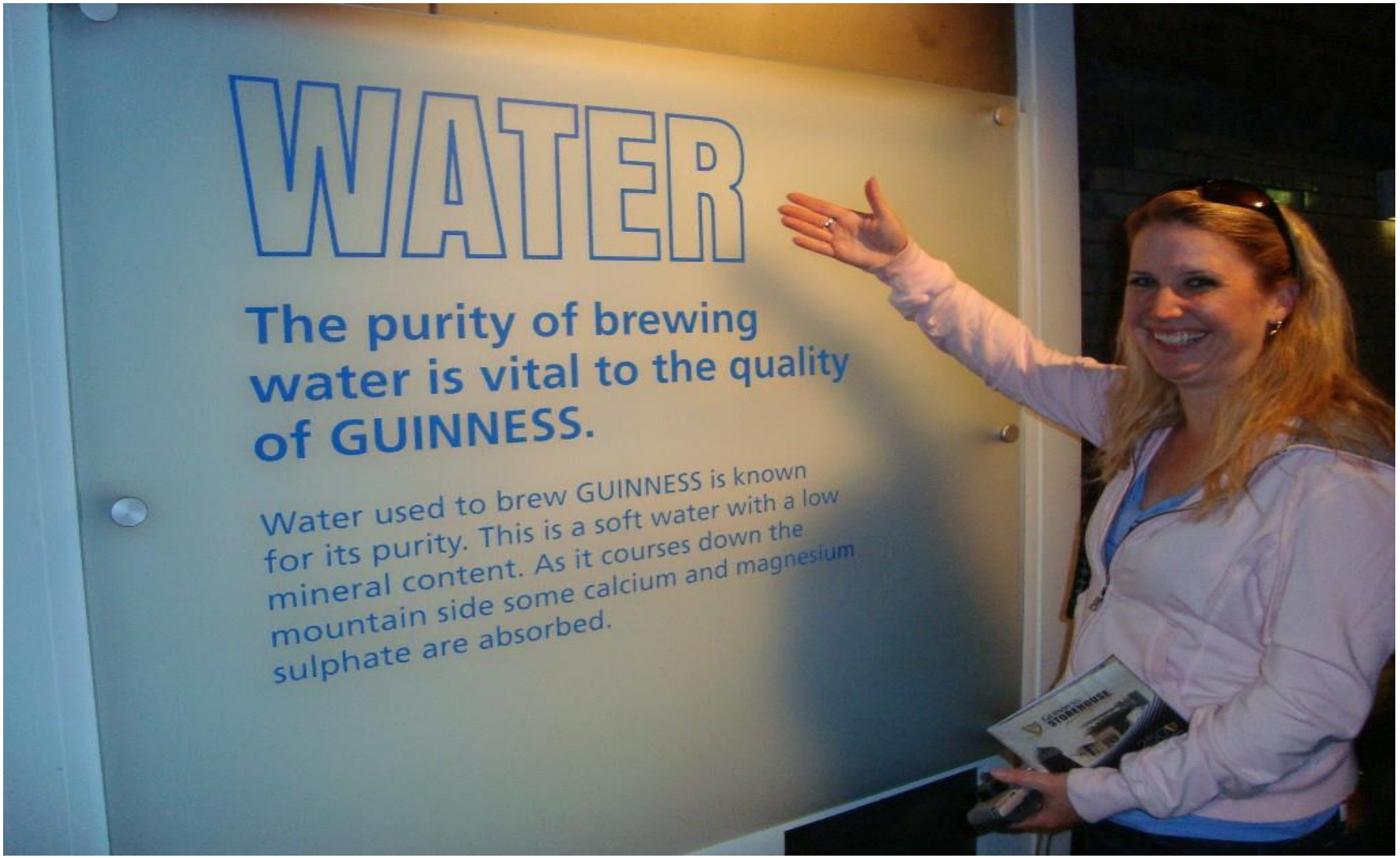
Why We Analyze Water.....



Thank you!

- Questions?