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Transmissive pH Probe Instructions

Overview

Ocean Optics' fully integrated pH systems provide full spectral analysis to help eliminate errors from dye leaching or from changes in turbidity, temperature, and ionic strength. Inherent calibration based on the physical properties of the immobilized indicator dye eliminates the need for frequent calibration. The ratiometric algorithm provides accurate and reproducible measurements at a high resolution.

The Transmissive pH Probe's form factor provides very fast response with high signal strength, though is limited to clear, non-turbid solutions. These sensors are compatible with aqueous solutions, ethanol/methanol solutions, ammonia, peroxides, and sodium hypochlorite solutions.

Transmissive Probes and Patches

The pH sensory substrates use a sol gel sensing material coated onto a transmissive patch. The immobilized indicator dye(s) are encapsulated into the sol gel matrix, allowing for the diffusion of ions while preventing leaching of the dye. The sensors provide very accurate measurements in the biological range, from pH 5 to 9. Advantages of these probes over traditional potentiometric devices include faster response time, easy storage and no maintenance, and low cost. These are especially useful for monitoring low conductivity samples such as boiler water, where electrode devices fail.

Software Interface

The Transmissive pH Probes can be used with a desktop system or the Jaz handheld spectrometer. The desktop system uses a special SpectraSuite software module that allows for easy calibration, convenient pH readings, customizable data logging, and comprehensive export of data and calibration values.

Transmissive pH Probe Benefits

- Chemistry
  - Proprietary Organically Modified Sol Gel (ORMOSIL) formulation engineered to maximize immunity to ionic strength sensitivity
  - Compatible with some organic solvents (i.e. acetone, alcohols, aromatics) ◊ Sol-Gel material chosen over typical polymer method, allowing for a faster response
  - time, versatility in the desired dopants, greater chemical compatibility, flexible coating, and enhanced thermal and optical performance
  - Indicator molecule allows high resolution measurement in biological range (pH 5-9)
- Simplified algorithm takes analytical and baseline wavelengths into account to reduce errors caused by optical shifts
- Sterilization
  - Transmissive patches are autoclavable
  - Transmissive patches are gamma and ethylene oxide sterilizable
- Maintenance
  - Long-term storage
  - Dry or wet storage
  - Single point reset
- Applications
  - Organic solvents
  - Low ionic strength cooling water
  - Biological Processes
  - Environmental
- Durable design allows probe to survive conditions/shocks that would cause glass electrodes to break

Fiber Optic pH Sensor System Components

The Fiber Optic pH Sensor system consists of the following:

- Transmissive pH probe (T300-UV/VIS or VIS/NIR)
- Ocean Optics VIS-NIR spectrometer (or Jaz Sensor module) that covers 350–1100 nm (slit options 25, 50, 100, 200)
- SpectraSuite Spectrometer Operating Software for reading values
- Light source (LS-1 Tungsten Halogen Light Source or a white LED)
- Transmissive pH patches, pack of 5 (PH-BCG-TRANS)

Calibration requires recording spectra in high and low pH samples, as well as in at least one pH standard solution (such as a NIST-traceable buffer).

For field measurements, the Ocean Optics handheld Jaz spectrometer offers an easy and portable solution. An SD card contains a script that allows you to use the factory calibration or a complete calibration, shows live pH values and provides the ability to save data right onto the SD card.

Probe Storage/Lifetime

Probes can be stored dry at room temperature for any amount of time. As they are used, the patches may slowly leach indicator dye from the sensing material. As a rule, **once the maximum absorbance at pH 11 falls below 0.1, the patch should be discarded and replaced** (assumes a reference of pH 1). The patch's lifetime depends on frequency of use, harshness of the samples it is exposed to, the temperature of samples, and other environmental factors.

Nature of Samples

The analyte solutions being measured should have a pH within the biological range (pH 5-9) for accurate readings. Data obtained from analyte solutions that register values above or below this range should not be considered valid within the specifications of the sensors. Aqueous solutions, ethanol/methanol solutions, peroxides, ammonia, and sodium hypochlorite solutions are all compatible with the sensor material. Samples being used with the transmissive probe should be optically transparent, having no turbidity or sediment present. It is also ideal to have analyte solutions that are colorless, though colored liquids can be compensated for.

Response time is dependent on the ionic strength of the solution, with higher salinity samples responding notably faster. For example, using the calibration buffers of pH 5 – 8 will show a 90% response in 10 seconds or less, but when pure D.I. water is being measured, more time is needed to equilibrate at a final value. Make sure that the probe is sufficiently submersed into the analyte solution, and that there are not bubbles present. To ensure no bubbles remain, shake the probe back and forth through the liquid to knock them free. Likewise, more accurate results will be obtained if the probe is rinsed once or twice with the analyte solution after calibration. This removes any residual buffer solution that may contaminate your sample.

When immersed in solution, the film dyes may leach very slowly over time and will have to be replaced. The film response rate is limited by diffusion of ions into the material; therefore increasing stirring speed and ionic strength tend to increase the response rate.

pH Probe Set Up

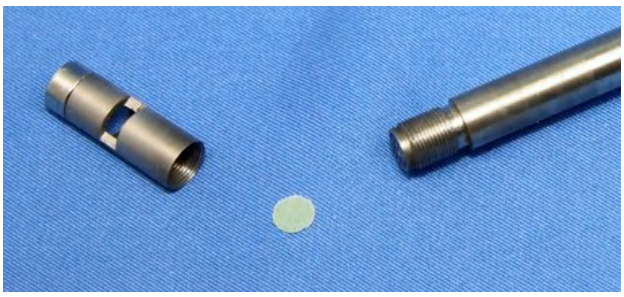
The following procedures describe how to connect and calibrate the transmissive pH probe using a VIS-NIR spectrometer, a light source and SpectraSuite software. See your spectrometer, light source and SpectraSuite manual for more detailed installation information (available at ***http://www.oceanoptics.com/technical/operatinginstructions.asp***).

Installing the pH Sensor System

► *Procedure*

Perform the steps below to install the pH Sensor components:

1. Install SpectraSuite software on your computer.
2. Connect the spectrometer to your computer using the supplied USB cable.
3. Install the light source as specified in its instructions.
4. Attach the fibers between the spectrometer, probe, and light source. The transmissive probe has two identical legs.
5. Turn on the light source and allow it to warm up for the period specified in the light source instructions.
6. The cap for the transmissive probe screws off. The probe uses a thin sensing film with adhesive on one side. This can be affixed to the lens of the probe. A second patch can be affixed to the mirror as well to dramatically increase signal strength and pH resolution.



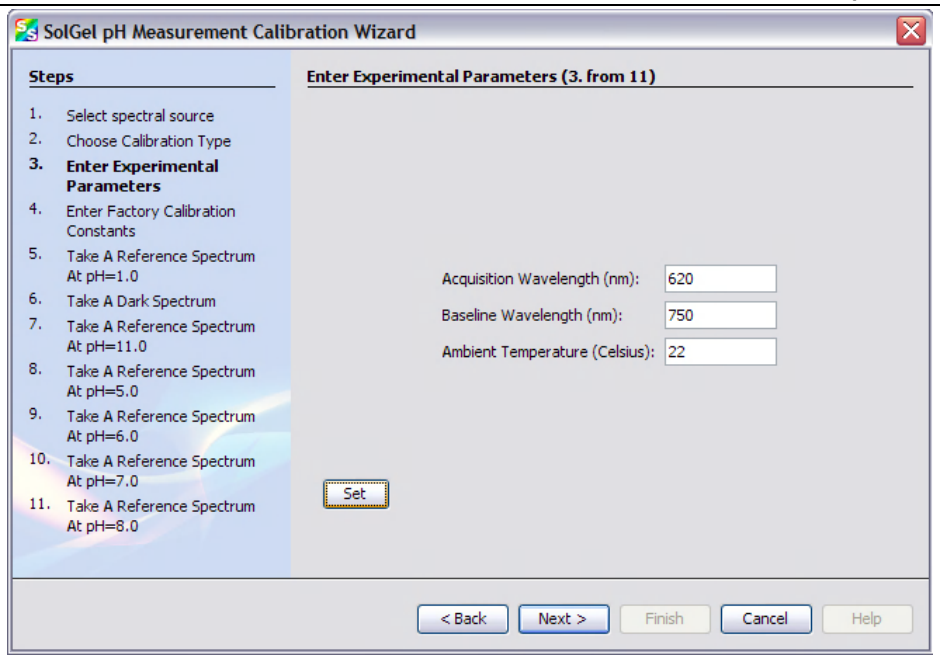
Calibrating the pH Sensor System

The pH sensor patches include a pre-calibrated pK value determined at the factory. This value was originally obtained at 22°C, and it is recalculated using the temperature compensation algorithm based on the temperature that was entered in SpectraSuite's Calibration Wizard. Using the Factory Calibration method is ideal for being able to start making pH measurements quickly, though it is less accurate than performing an Independent Calibration. The specifications listed for the probes assume a complete Independent Calibration, as this eliminates the errors seen from temperature and other environmental differences.

Using Factory Calibration

► *Procedure*

1. Open SpectraSuite and select **File | New | New Sol Gel pH Measurement**.
2. Click the Calibration Wizard button to begin the calibration.
3. Select the spectrometer to use and click **Next**.
4. Select **Use Factory Calibration**. Click the box for **Using Reflective Probe,** and then click **Next**. The **Enter Experimental Parameters** screen appears.



5. Enter your Experimental parameters: **Acquisition Wavelength**, **Baseline Wavelength**, and approximate **Ambient Temperature**. Click **Set**, then click **Next.**

Note

For pH probes that perform in the biological range (pH 5 – 9), the **Acquisition Wavelength** is **620nm** and the **Baseline Wavelength** is **509nm**.

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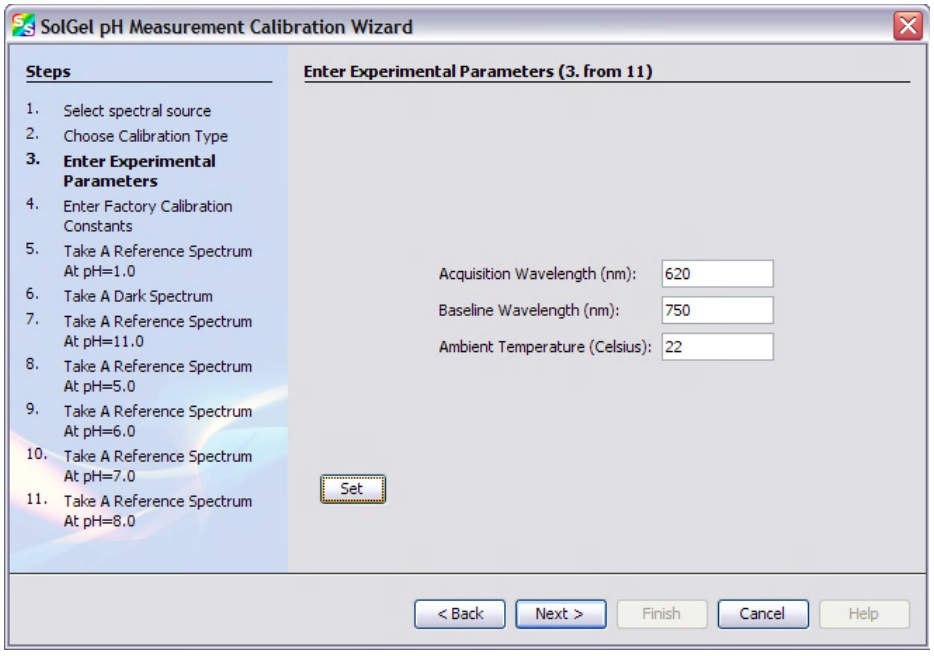
6. Enter the value for pK that came with your probe pH patch. Then click **Next**.
7. Take a low pH reference spectrum at pH 1.0. To do this,
   a. Submerge the probe tip in pH 1 buffer and shake back and forth to remove bubbles.
   b. Wait 30 seconds, then click **Acquire**. The spectrum shown should have a maximum intensity around 80% of the saturation level. Most Ocean Optics spectrometers have a saturation limit of 4000 counts. If saturation is occurring, reduce the integration time appropriately. If the signal is too low, increase the integration time until the intensity at 620nm is at least over 1000 counts.
   c. You can click **Acquire** repeatedly to update the spectrum after adjusting the integration time; the last time you press the button will be the last reference that is saved.
   d. Once the signal looks strong, make sure you've updated the acquisition and then click **Next**.
8. Take a dark spectrum. To do this, block the light source and click **Acquire Dark Spectrum**. Then click **Next**. Be sure to allow enough time for the system to complete an entire scan while in the dark before acquiring the spectrum.
9. Unblock the light source.

10. Take a high reference spectrum for pH 11.0. To do this, submerge the probe tip in pH 11 buffer and shake back and forth to remove bubbles. It's a good idea to rinse the probe with buffer before submersion to ensure residual buffer from before does not contaminate your sample. Allow 30 seconds, and then click **Acquire**. When complete, click **Next**.
11. Depending on the value for pK you previously entered, the wizard will ask you to expose the probe to either pH 5 or pH 8. For pK values less than 6.5, pH 8 is used; for pK value greater than 6.5, pH 5 is used. Submerge the probe tip in the requested buffer and shake back and forth to remove bubbles. Allow 30 seconds, click **Acquire**, and then click **Finish**.
12. You are now ready to take pH measurements. See *Taking pH Measurements*.

Performing an Independent Calibration

► *Procedure*

1. Open SpectraSuite and select **File | New | New Sol Gel pH Measurement**.
2. Click the **Calibration Wizard** button to begin the calibration.
3. Select the spectrometer to use and click **Next**.
4. Select **Perform Independent Calibration**. Click the box for **Using Reflective Probe**, and then click **Next**. The **Enter Experimental Parameters** screen appears.
5. Enter your Experimental parameters: **Acquisition Wavelength**, **Baseline Wavelength**, and approximate **Ambient Temperature**. Click **Set**, then click **Next.**



Note

For pH probes that perform in the biological range (pH 5 – 9), the **Acquisition Wavelength** is **620nm** and the **Baseline Wavelength** is **509nm**.

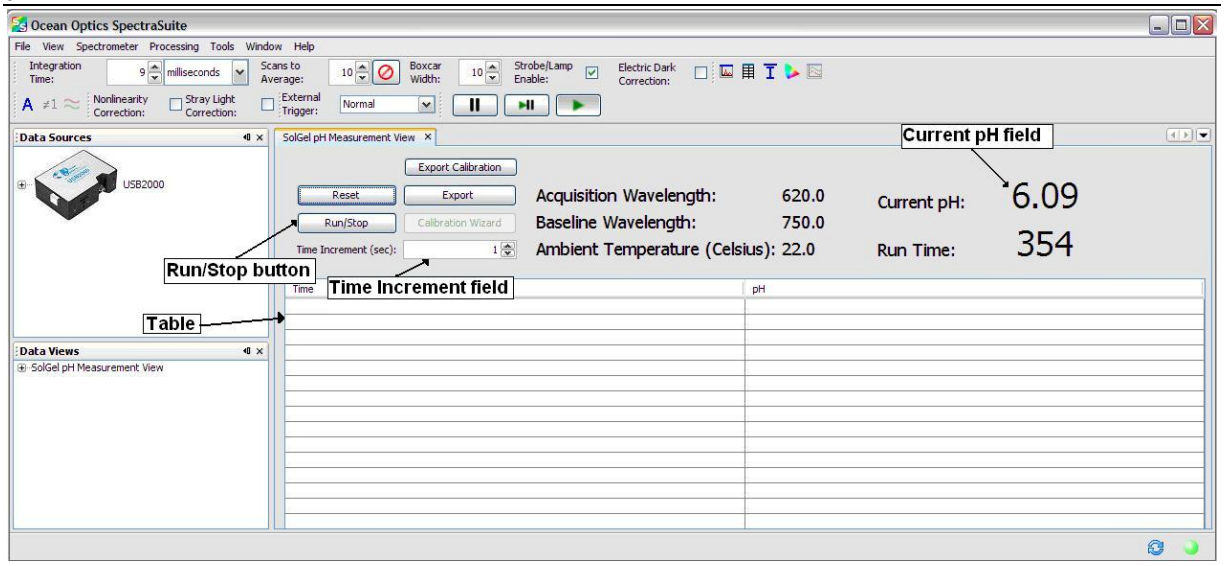
6. Take a low pH reference spectrum at pH 1.0. To do this,
   a. Submerge the probe tip in pH 1 buffer and shake back and forth to remove bubbles.
   b. Wait 30 seconds, then click **Acquire**. The spectrum shown should have a maximum intensity around 80% of the saturation level. Most Ocean Optics spectrometers have a saturation limit of 4000 counts. If saturation is occurring, reduce the integration time appropriately. If the signal is too low, increase the integration time until the intensity at 620nm is at least over 1000 counts.
   c. You can click **Acquire** repeatedly to update the spectrum after adjusting the integration time; the last time you press the button will be the last reference that is saved.
   d. Once the signal looks strong, make sure you've updated the acquisition and then click **Next**.
7. Take a dark spectrum. To do this, block the light source and click **Acquire Dark Spectrum**. Then click **Next**. Be sure to allow enough time for the system to complete an entire scan while in the dark before acquiring the spectrum.
8. Unblock the light source.
9. Take a high reference spectrum for pH 11.0. To do this, submerge the probe tip in pH 11 buffer and shake back and forth to remove bubbles. It's a good idea to rinse the probe with buffer before submersion to ensure residual buffer from before does not contaminate your sample. Allow 30 seconds, and then click **Acquire**. When complete, click **Next**.
10. Follow the wizard and repeat Step 9 for pH buffers 5, 6, 7, and 8 (follow on-screen prompts). Again, it is a good idea to rinse the probe tip with buffer before submersion to prevent sample contamination. Then, click **Finish.**
11. You are now ready to take pH measurements.

Taking pH Measurements

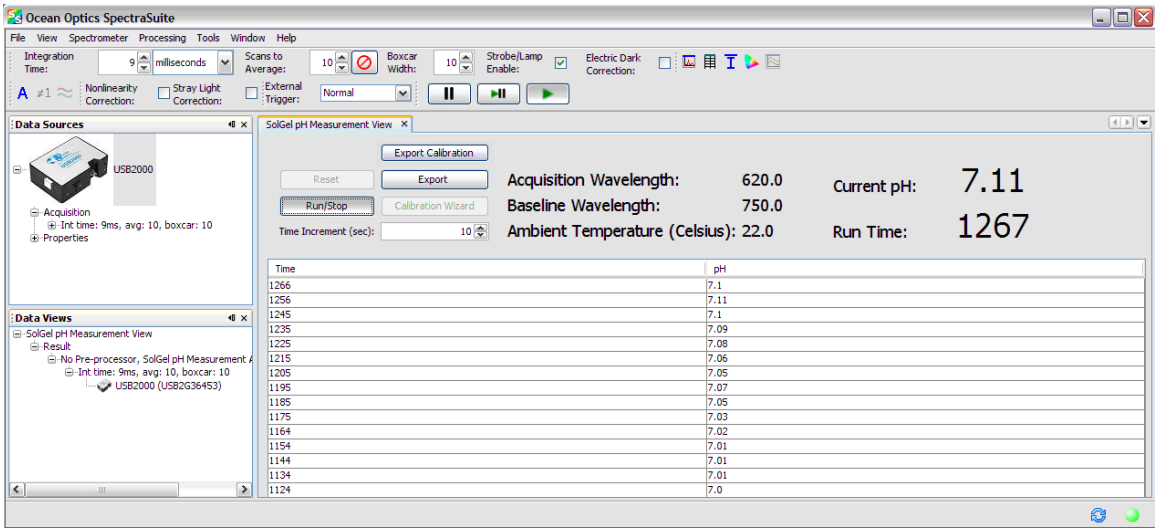
Now that you have finished calibrating your pH sensor system, you can take pH measurements in the biological range.

► *Procedure*

1. Submerge the probe tip into analyte solution for pH measurement in the biological range. The pH value appears on the screen in the **Current pH** field (upper right corner).



2. Click the **Run/Stop** button to toggle data acquisition appearing in the lower table on the screen. Data is recorded at the time interval you specify in the **Time Increment (sec)** field.



3. Click one or all of the following buttons:

   - **Reset:** Clears the table and restarts the run time.
   - **Export:** Opens a window to save your data in a format that can be opened with Microsoft Excel or a text program such as WordPad. The exported data file contains all of the variables that you have entered and have been calculated, along with a time stamp for data acquisition and save, the time-resolved pH data, and complete spectra for all reference and calibration buffers used.
   - **Export Calibration**: Opens a window to save your calibration data. This creates a file containing the reference spectra and other variables that can later be loaded via the Calibration Wizard, allowing for very quick setup.

Algorithms Used

pH Calculation

$$pH = pK + Slope * \log\left(\frac{Abs_{Sample}}{Abs_{pH11} - Abs_{Sample}}\right)$$

…where Abs_{Sample} is the sample absorbance at 620nm with baseline correction, and Abs_{pH11} is the absorbance at pH 11 at 620nm with baseline correction.

Temperature Compensation

When you select **Use Factory Calibration** in SpectraSuite, the value for pK is adjusted via the van't Hoff equation based on the current temperature you entered:

$$pK_2 = pK_1 + \log\left(e^{-480*\left(\frac{1}{T_2}-\frac{1}{T_1}\right)}\right)$$

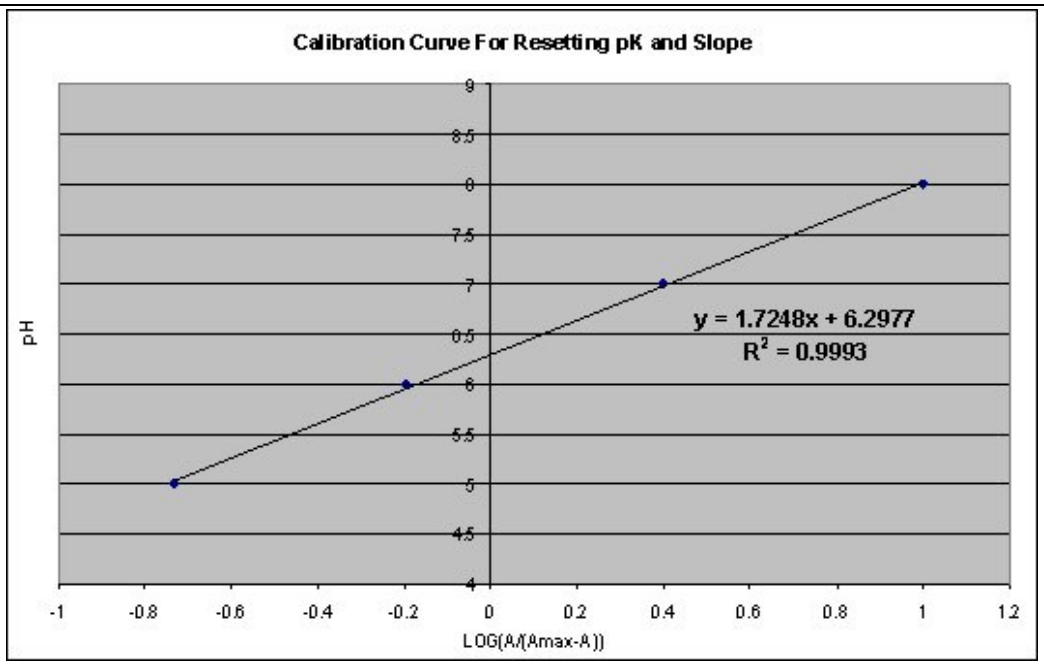
$$pH_2 = pH_1 + \log\left(e^{-480*\left(\frac{1}{T_2}-\frac{1}{T_1}\right)}\right)$$

Resetting pK and Slope

An x-y plot is made using data obtained from intermediate buffers 5 through 8. The x-axis is of the term:

$$\log\left(\frac{Abs_{Sample}}{Abs_{pH11} - Abs_{Sample}}\right)$$

…for each of the buffers. The y-axis shows the pH value of the buffers. This generates a plot such as the one shown below:



Performing a linear fit gives a line with pK equal to the y-intercept and slope equal to the slope. In the example chart above, the new pK value would be 6.2977 and the new slope value would be 1.7248.

Specifications

Specification	Ocean Optics pH Probe Value
pH Range	5 – 9
Temperature Range	-5 – 80°C
Accuracy	<1% of reading across range
Resolution	0.02 pH
Response Time (t_{90})	30 seconds Response time increases with decreasing ionic strength
Calibration Requirements (minimum)	3 buffers
Sterilization	Gamma, EtO, Autoclave
Chemical Compatibility	Aqueous, alcohols, some organic solvents, peroxides, ammonia, sodium hypochlorite
Chemicals to Avoid	Concentrated acids
Drift (Continuous Stability)	1% per day
Discrete Stability (Lifetime)	50 uses or more, dispose when absorbance at pH 11 < 0.1 (assumes pH 1 reference)
Storage Conditions	Dry or wet storage