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Smart pH Cuvettes Instructions

Overview

The Fiber Optic pH Sensor system consists of the following:

- Smart pH Cuvettes (1 cm x 1 cm PMMA or quartz) and/or patches
- Ocean Optics VIS-NIR spectrometer (or Jaz Sensor module)
- SpectraSuite software for reading values
- Light source (LS-1 Tungsten Halogen Light Source with a blue filter or a white LED)
- CUV-UV Cuvette Holder
- Connecting Fibers

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).

The Smart pH Cuvettes use a sol gel sensing material coated onto the inner walls of a cuvette. 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 cuvettes provide very accurate measurements in the biological range, from pH 5 to 9. Advantages of these cuvettes 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.

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.

Smart pH Cuvettes are 1 cm by 1 cm and are made of Poly(methyl methacrylate) (PMMA) or quartz. The PMMA material has a temperature range of -5 °C to 70 °C, while quartz is available for specific applications such as high-temperature measurements. The cuvettes are compatible with aqueous solutions, ethanol/methanol solutions, ammonia, peroxides, sodium hypochlorite solutions, while the quartz cuvettes are also compatible with concentrated acids (nitric, sulfuric, acetic, etc) and acetone.

Although PMMA cuvettes are designed to be disposable, they can be used multiple times. The lifetime depends on the extent of exposure to high pH levels and harsh chemicals. See *Cuvette Storage/Lifetime* for more information.

Cuvette Storage/Lifetime

Smart Cuvettes can be stored dry at room temperature for any amount of time. As they are used, the cuvettes may slowly leach indicator dye from the sensing material. As a rule, **once the maximum absorbance at pH 11 falls below 0.1, the cuvette should be discarded and replaced** (assumes a reference of pH 1). The cuvette'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 cuvette. Concentrated acids and acetone will ruin the plastic cuvettes, so these types of chemicals should be avoided. Aqueous solutions, ethanol/methanol solutions, peroxides, ammonia, and sodium hypochlorite solutions are all compatible with the sensor material and the plastic cuvette. Samples 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 cuvette is filled to a sufficient level with the analyte solution. If the liquid level is at or below the optical path, the data will not be valid. To ensure there is sufficient liquid, it is recommended to fill the cuvette about 70% its height. Likewise, more accurate results will be obtained if the cuvette is rinsed once or twice with the analyte solution after calibration. This removes any residual buffer solution that may contaminate your sample. Note that once the cuvette has been affixed into the cuvette holder, it should not be moved or removed until all measurements have been completed.

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 Smart Cuvette Set Up

The following procedures describe how to connect and calibrate pH Smart Cuvettes using a VIS-NIR spectrometer, a light source and SpectraSuite software. See your spectrometer and SpectraSuite manual for more detailed installation information.

Installing the pH Sensor System

► *Procedure*

Perform the steps below to install the pH Sensor components:

1. Install SpectraSuite 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, cuvette holder, and light source.

5. Turn on the light source and allow it to warm up for the period specified in the light source instructions.

Caution

Make sure that the cuvette says fastened in the cuvette holder with the tightening screw and that it does not move until all measurements have been completed. Any movement will change the optical signal, disrupting the quality of the measurement.

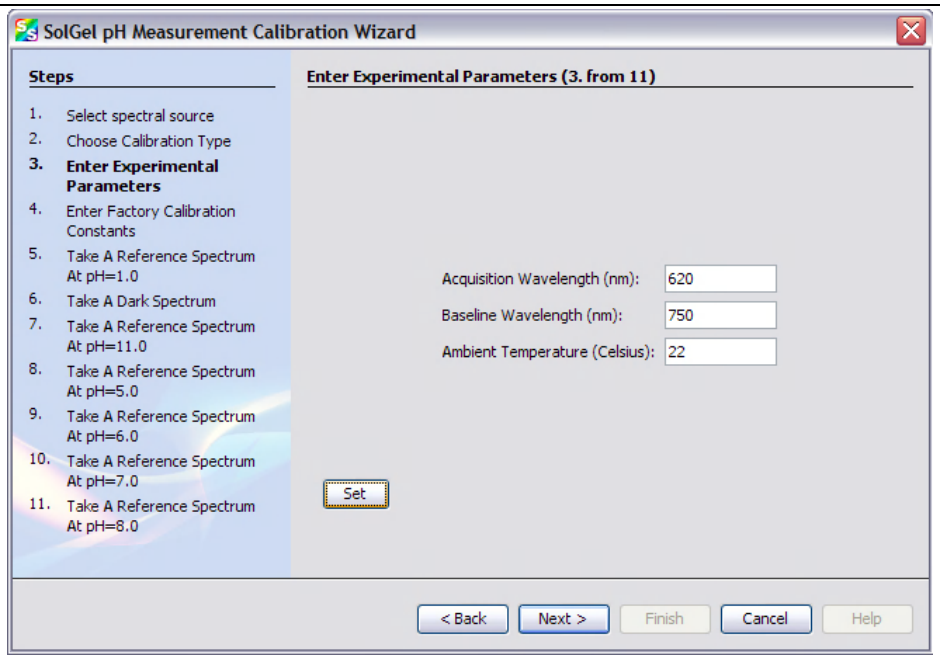
Calibrating the pH Sensor System

The Smart Cuvettes 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 cuvettes 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** and click **Next**. The Experimental Parameters screen appears.



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

Note

For Smart pH Cuvettes that perform in the biological range (pH 5 – 9), the **Acquisition Wavelength** is 620nm and the **Baseline Wavelength** is 750nm.

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6. Enter the value for pK that came with your Smart pH Cuvette. Then click **Next**.
7. Take a low pH reference spectrum at pH 1.0. To do this, fill the cuvette with pH 1 buffer. Allow it to sit for 5 seconds, then remove the buffer. Refill the cuvette with a fresh sample. Click **Acquire**. The program adjusts the integration time to prevent saturation. When complete, click **Next**.
8. Take a dark spectrum. To do this, block the light source and click **Acquire Dark Spectrum**. Then click **Next**.
9. Unblock the light source.
10. Take a high reference spectrum for pH 11.0. To do this, remove the pH 1 buffer and fill the cuvette with pH 11 buffer. Allow this to sit for 10 seconds, then remove the buffer and refill the cuvette with a fresh sample. When complete, click **Next**.
11. Depending on the value for pK you previously entered, the wizard will ask you to add pH 5 or pH 8 buffer. For pK values less than 6.5, pH 8 is used; for pK value greater than 6.5, pH 5 is used. Remove the pH 11 buffer and fill the cuvette with the requested pH buffer. Allow it to sit for 10 seconds, remove the buffer and refill the cuvette with a fresh sample. 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**. Select **Perform Independent Calibration** and click **Next**. The Experimental Parameters screen appears.
4. Enter your Experimental parameters: **Acquisition Wavelength**, **Baseline Wavelength**, and approximate **Ambient Temperature**. Click **Set**, then click **Next.**

Note

For Smart pH Cuvettes that perform in the biological range (pH 5 – 9), the **Acquisition Wavelength** is 620nm and the **Baseline Wavelength** is 750nm.

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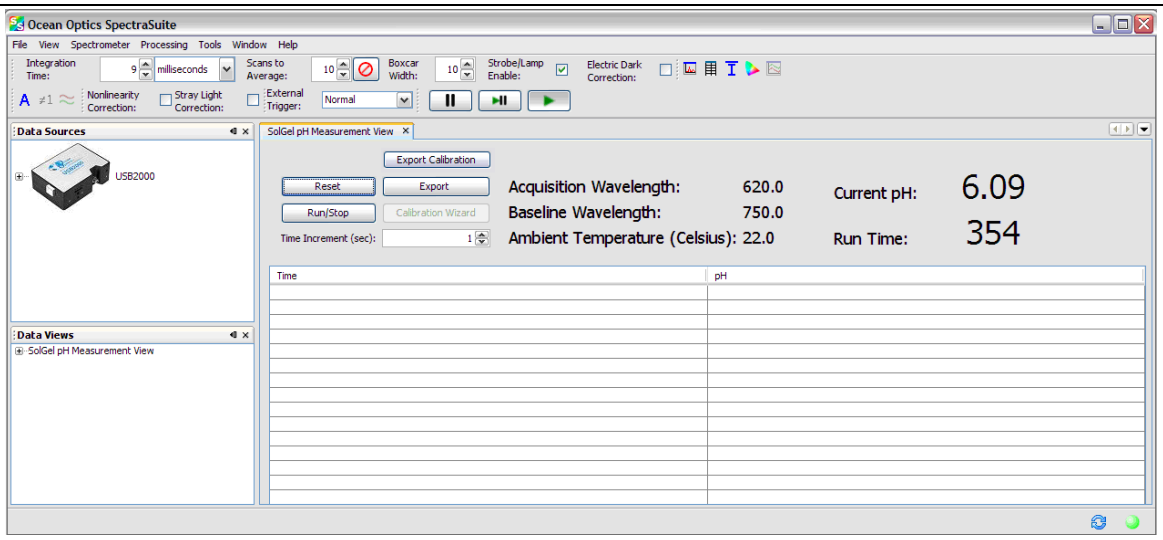
5. Take a low pH reference spectrum at pH 1.0. To do this, fill the cuvette with pH 1 buffer. Allow it to sit for 5 seconds, then remove the buffer. Refill the cuvette with a fresh sample. Click **Acquire**. The program adjusts the integration time to prevent saturation. When complete, click **Next**.
6. Take a dark spectrum. To do this, block the light source and click **Acquire Dark Spectrum**. Then click **Next**.
7. Unblock the light source.
8. Take a high reference spectrum for pH 11.0. To do this, remove the pH 1 buffer and fill the cuvette with pH 11 buffer. Allow this to sit for 10 seconds, then remove the buffer and refill the cuvette with a fresh sample. When complete, click **Next**.
9. Follow the wizard and repeat Step 8 for pH buffers 5, 6, 7, and 8 (follow on-screen prompts). Then, click **Finish.**
10. You are now ready to take pH measurements. See Taking pH Measurements.

Taking pH Measurements

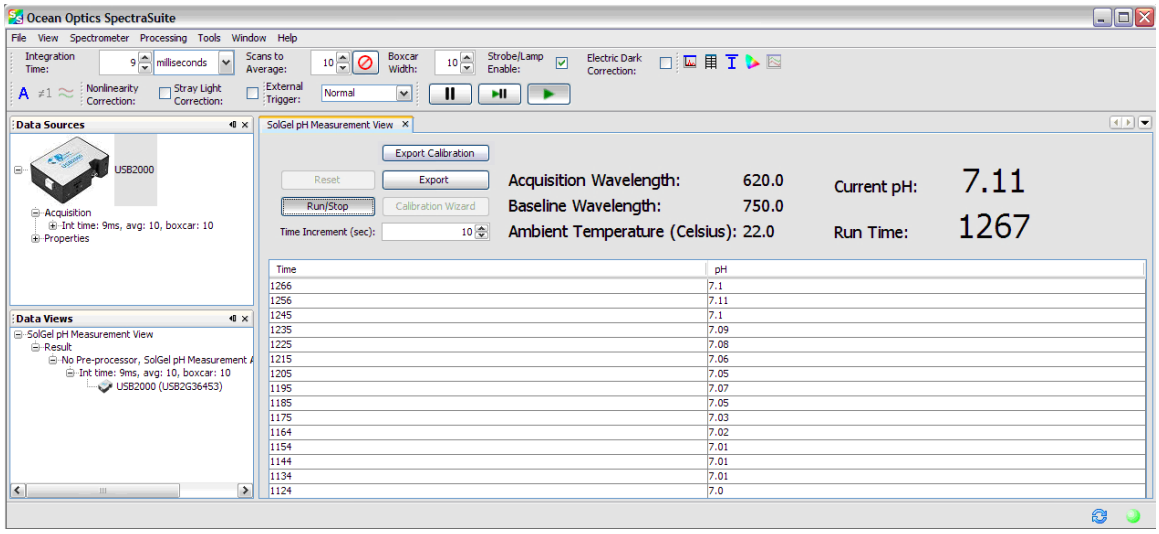
Now that you have finished calibration, you can take pH measurements in the biological range.

► *Procedure*

1. Fill the cuvette with the 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. Press 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 Interval (sec)** field.



3. Click the **Reset** button to clear the table and restart the run time.
4. Click the Export button to open 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.
5. Click the Export Calibration button to open a window to save your calibration data. This will create 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:

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

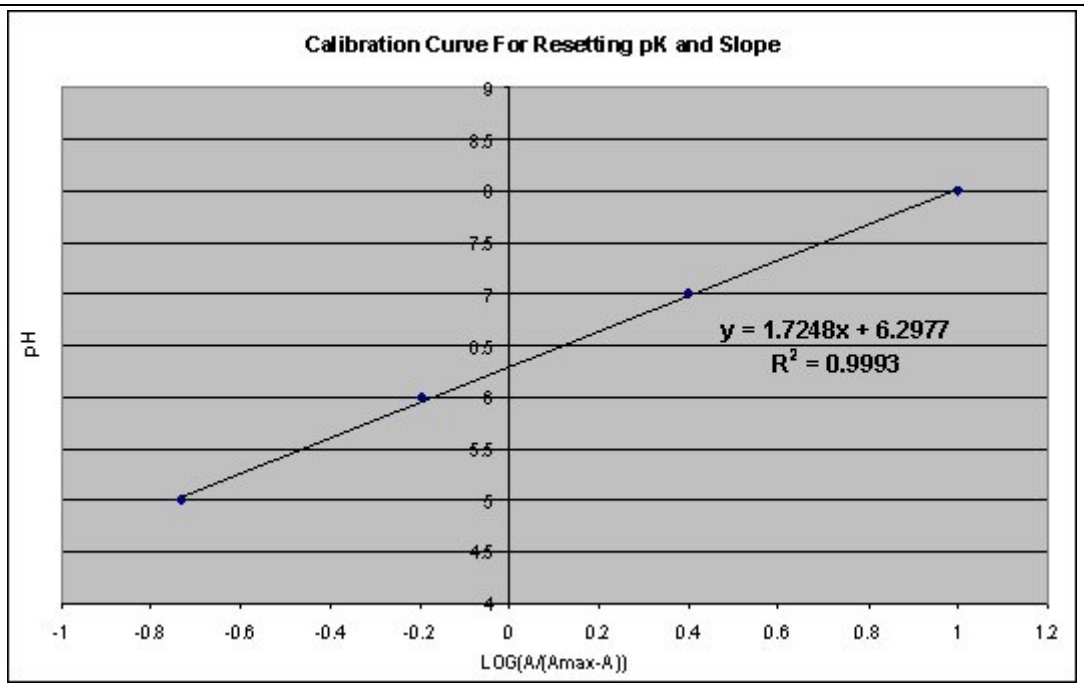
$$pH_2 = pH_1 + \log\left(e^{-480*\left(\frac{1}{T_2}-\frac{1}{T_1}\right)}\right)$$

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	Value
Size and Materials	1 cm x 1 cm PMMA cuvette; 1 cm x 1 cm quartz cuvettes available for specific applications such as high-temperature measurements
pH Range	Biological range (5-9)
Temperature range	-5 to 70 °C for PMMA cuvettes
Accuracy	<1% of reading
Resolution	0.01 pH
Response Time	90% step response in 10 seconds
Standardization	User needs 2 buffers (pH 1 and 11)
Factory Calibration	User needs 1 buffer (pH 5 or 8)
User Complete Calibration Option	Users have the option to perform their own complete calibration, requiring 4 buffers (pH 5, 6, 7, and 8)
Sensory Signal	Absorbance at 620nm and 750nm
Expendable Parts	PMMA cuvettes are semi-disposable, -- they can be used multiple times, but they are not intended for long-term permanent use

Specification	Value
Usage Lifetime	Potential of being used as many as 50 or more measurements. Lifespan depends on extent of exposure to high pH levels and harsh chemicals. Discard after maximum absorbance at pH 11 falls below 0.1 (assumes reference of pH 1).
Recommended Spectrometer Configuration	Any VIS/NIR spectrometer can be used. Either LS-1 (with blue filter) or white LED can be used as light source.
Temperature Compensation	Factory calibrated pK values are adjusted based on the user-inputted temperature via the van't Hoff equation (see Algorithms Used). Factory coefficients are generated at 22°C.
Chemical Compatibility: PMMA Glass	 Aqueous solutions, ethanol/methanol solutions, ammonia, peroxides, sodium hypochlorite solutions All listed for PMMA plus concentrated acids (nitric, sulfuric, acetic, etc) and acetone
Chemicals to Avoid for PMMA	Concentrated acids (nitric, sulfuric, acetic, etc), acetone
Sterilization: PMMA Glass	 Gamma irradiation and Ethylene Oxide Autoclavable, Gamma irradiation, and Ethylene Oxide TBD