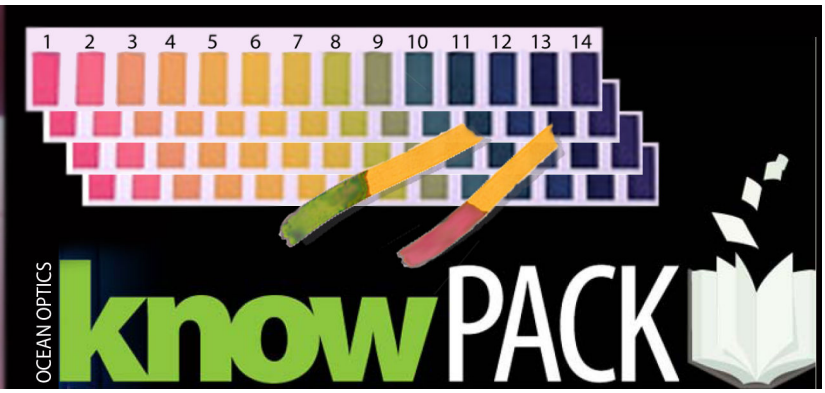

Transmissive pH Sensing in Microtiter Wells

GMP

Introduction

Our newest transducer materials are designed to encapsulate pH dyes for optical pH sensing. These materials can be applied to various sampling devices and combined with fiber optic spectrometers and accessories for convenient, non-intrusive monitoring. The pH sensor has been developed for monitoring samples within the pH range of 5-9 (biological range). The proprietary sol-gel matrix doped with bromocresol green dye (BCG) serves as an excellent material for optical pH measurements.

Thru the use of a mathematical algortithm the sensor can detect pH changes down to 0.01 pH units. Smart pH Cuvettes are semi-disposable and require very little maintenance. The Sol-Gel BCG has been coated inside microtiter plates for the detection and continuous monitoring of pH changes over the biological range as a proof of concept to be used for the monitoring of cellular pH environments.

Case Study

Transparent plastic microtiter wells were affixed with a thin adhesive PMMA material coated earlier with Smart pH sol-gel formulation at the bottom of the wells. This allows for transmissive pH sensing of the analyte solutions placed into the wells. These wells come together as a plate of many, and are moved via a robotic positioning system to align them with the light source and recovery. Our customer's current configuration uses photodiode detectors and light filters to observe absorbance at the necessary wavelengths.

As a quick feasibility study, a brief titration was performed using two buffers only: pH 1 (absorbance reference) and pH 11 (observed). The pH 11 buffer produced a strong, though distorted, absorbance curve very similar to what is seen for the Smart pH Cuvettes, which showed that these wells had the potential to work as hoped (*Figure 1*.).

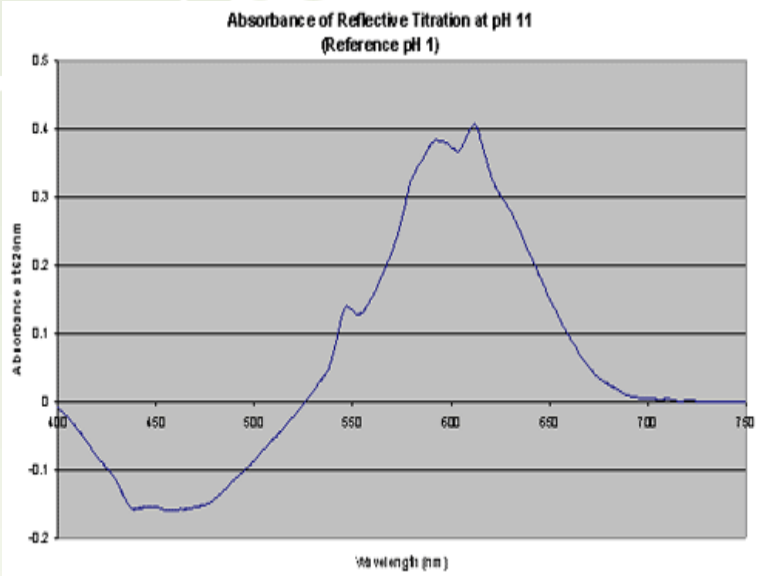

Figure 1. Absorbance of Reflective Titration at pH 11

This was performed using a standard cuvette holder turned vertically, with collimating lenses on each side. Light was fed to the top of the cuvette holder from an LS-1 light source with a blue filter via a P200 UV/VIS fiber. The signal was then sent through a P300 UV/SR fiber to a USB2000 with grating #2, 200um slit, and no lens installed. The experimental setup is shown in *Figure 2*.

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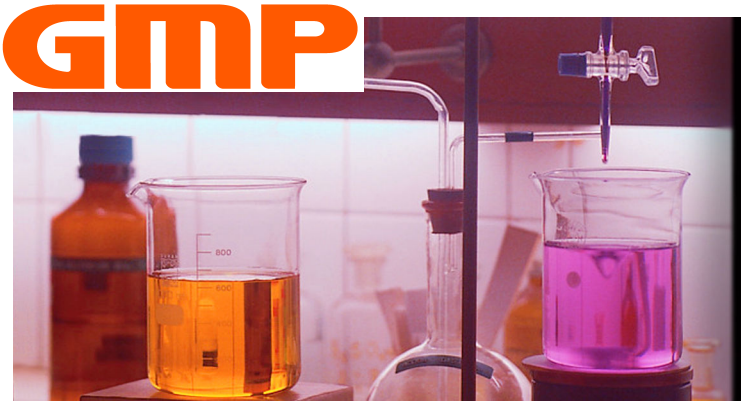

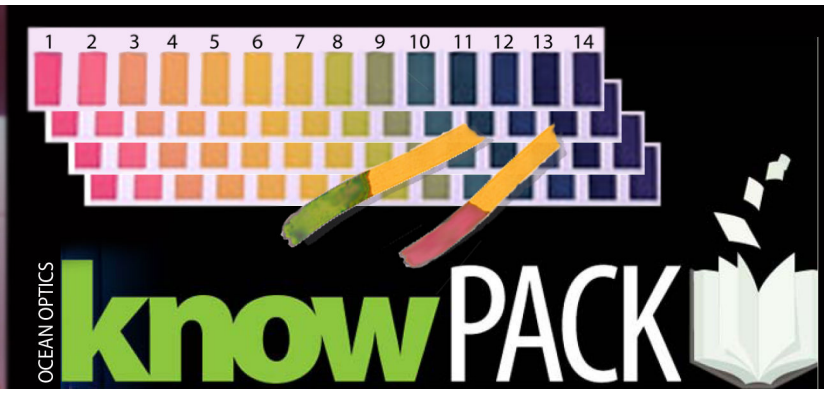

Figure 2. Experiment setup

However, when our customer carried out their own set of titrations, the data did not adhere to the pH algorithm whatsoever; everything appeared very randomized. (See *Figure 3*.)

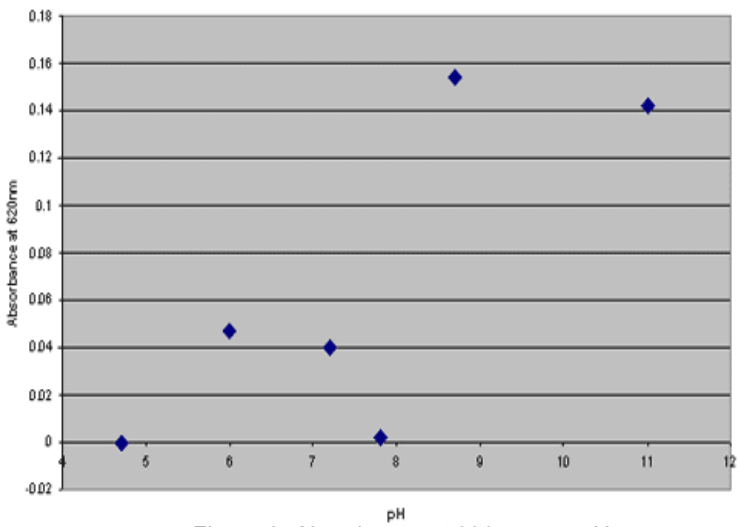

Figure 3. Absorbance at 620 nm vs. pH

As a result, a full titration was performed here at Ocean Optics to determine the cause of this significant error. The same fibers, light source, spectrometer, and general setup were used as previously described. Two issues were determined to be the most probable causes of the poor data seen by our customer; these included the lack of collimating lenses being used at the facility, as well as an incorrect baseline correction wavelength. They were previously instructed to use 750nm as the baseline correction found in the algorithm, though they used 880nm due to availability of that filter. The tests performed here showed that neither 750nm or 880nm were acceptable baseline wavelengths, rather the ideal wavelength to use was 509nm. Results from the full titration are shown in *Figure 4* and *Figure 5*.

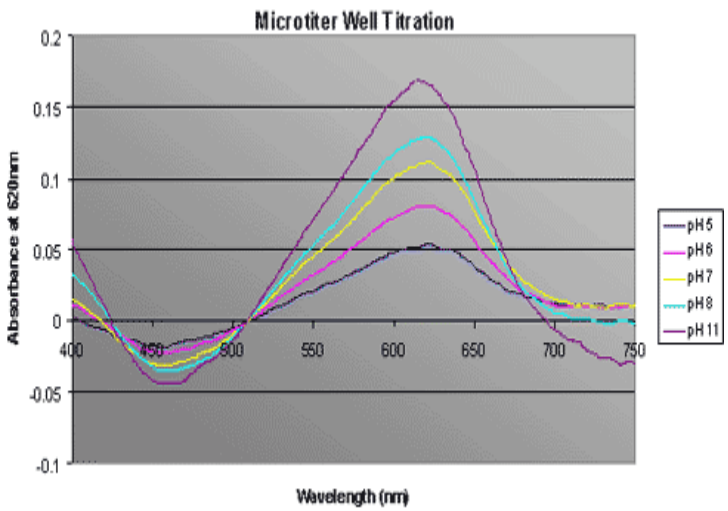

Figure 4. Absorbance at 620 nm vs. Wavelength (nm)

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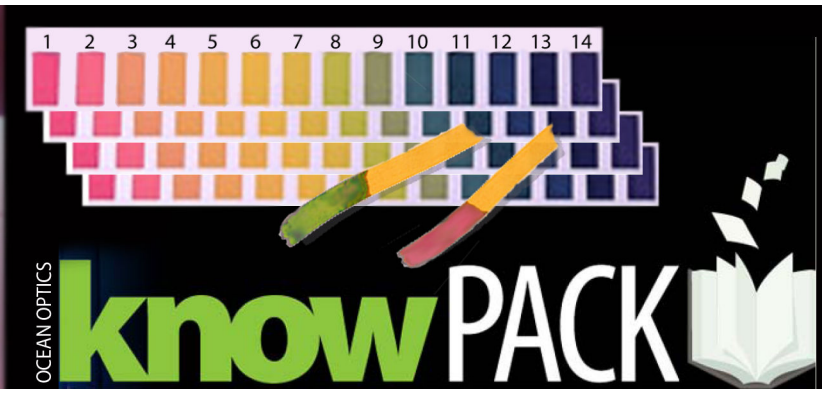

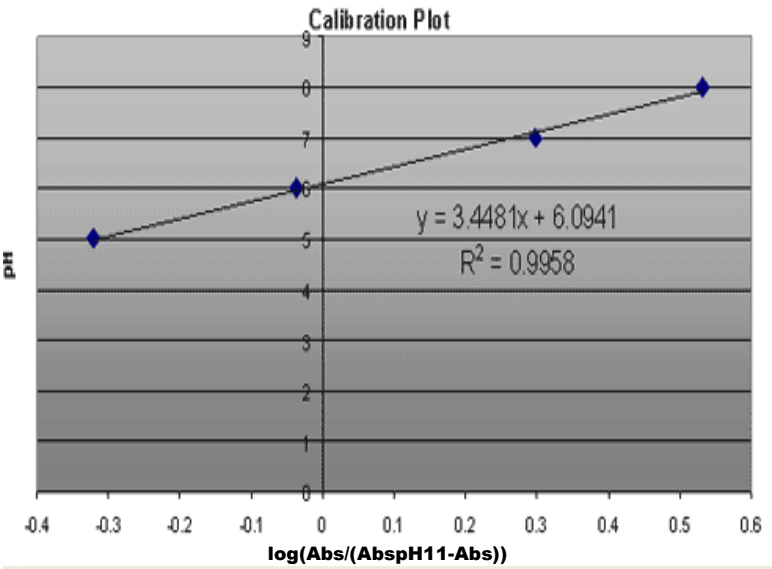

Figure 5. Calibration Plot

This has shown that the pH sensor affixed to the bottom of microtiter wells can accurately monitor and report the pH of transmissive analyte solutions.

Algorithm used:

$$pH = pK + Slope * \log\left(\frac{A_{620nm} - A_{509nm}}{\left(A_{pH11,620nm} - A_{pH11,509nm}\right) - \left(A_{620nm} - A_{509nm}\right)}\right)$$

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