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A Hue-Based Method for pH Determination

Blair Pearson

Kelly Ramzy

Ryan Bergio

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Senior Honors Project
Performed under the visage of Kevin Cantrell
In concert with Kelly Ramzy and Ryan Bergio

Background

Matlab is a matrix-based programming environment that can effectively handle large quantities of data at once. It includes the potential for image processing, but also for connecting devices over a serial port – essentially, anything that may connect via USB port. Arduino boards are small, prototyping motherboards with open-source programming software. The connection between Arduino and Matlab is supported; in this regard, Arduino provides a convenient means to carry information from Matlab code to a custom-built circuit or device.

As pH is one of the most ubiquitous data in chemistry, there are a variety of technologies that measure it. The first and most common is the pH electrode (Fig. 1): this is a probe that measures H^+ activity with a membrane against a reference electrode of known reactivity. The probe returns a voltage proportional to that activity to a designated meter, which converts the value to pH. The delicate membrane must be hydrated before use, and the electrode/meter setup must be recalibrated (preferably every two hours, though there is some variation between models). Electrodes range in price, size, and precision, but all require physical contact with the sample, and a minimum sample volume.

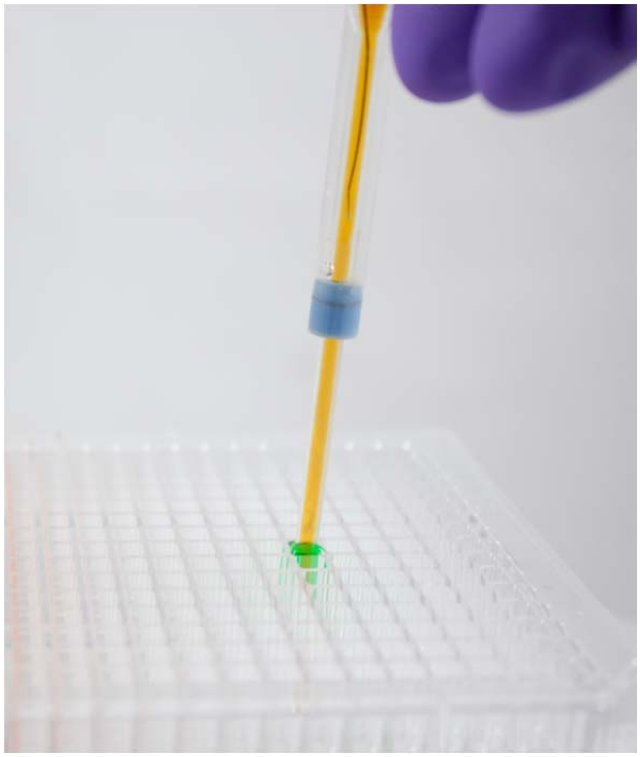

Figure 1: Ross Micro pH Electrode

Other methods include spectroscopic means of determining pH. These use an indicator molecule that changes color depending on the pH of its solution. Shining light through the solution and measuring the absorbance of that light at varying wavelengths provides an extremely precise way to measure exactly what color the solution is. These spectra can be related to the solution's pH very precisely (Fig. 2). However, this technique requires a tremendous amount of time, necessitates a reasonably large sample volume, and uses rather expensive equipment.

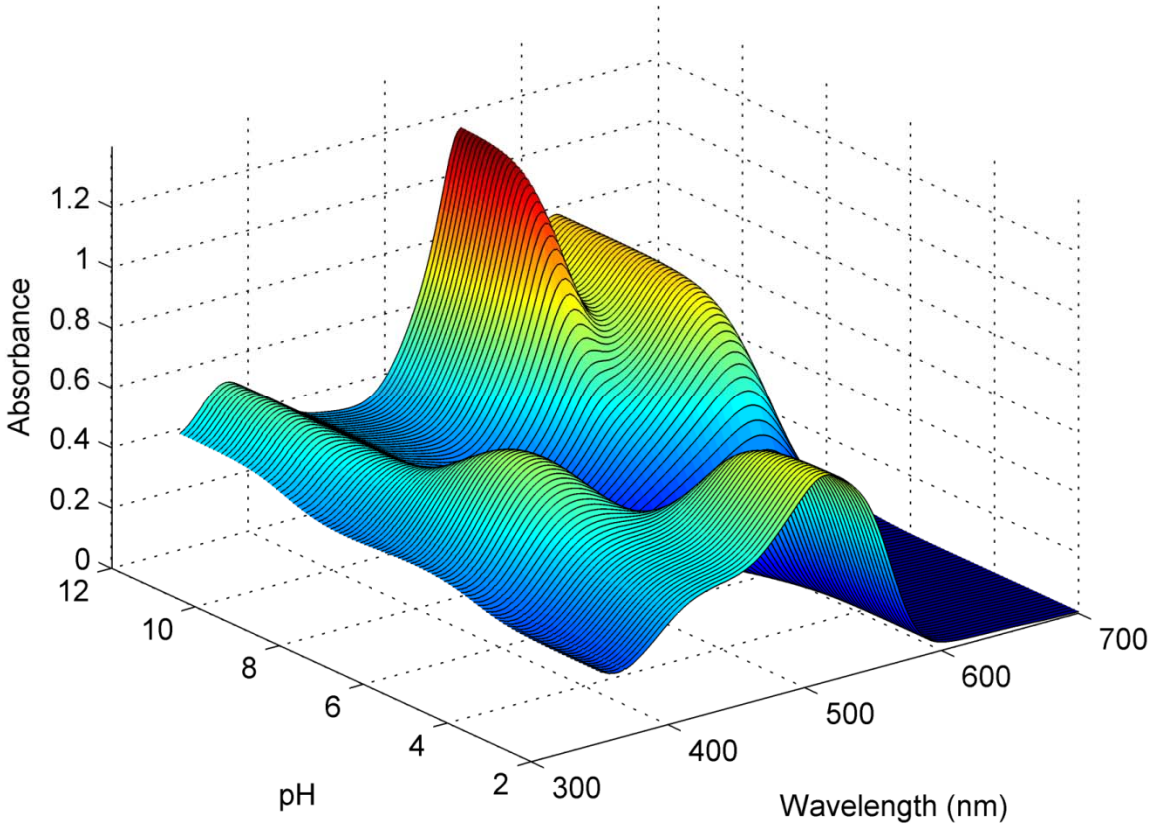

Figure 2: Absorbance Spectra for a pH Indicator

Furthermore, most indicator molecules only change pH over a rather narrow region. To compensate for this, a universal indicator can be made by combining a number of indicator molecules that change color over different regions (Fig. 3). Again, a relationship between the pH and the solution's absorbance spectra is well defined, but measuring that spectrum is time-intensive and requires expensive equipment.

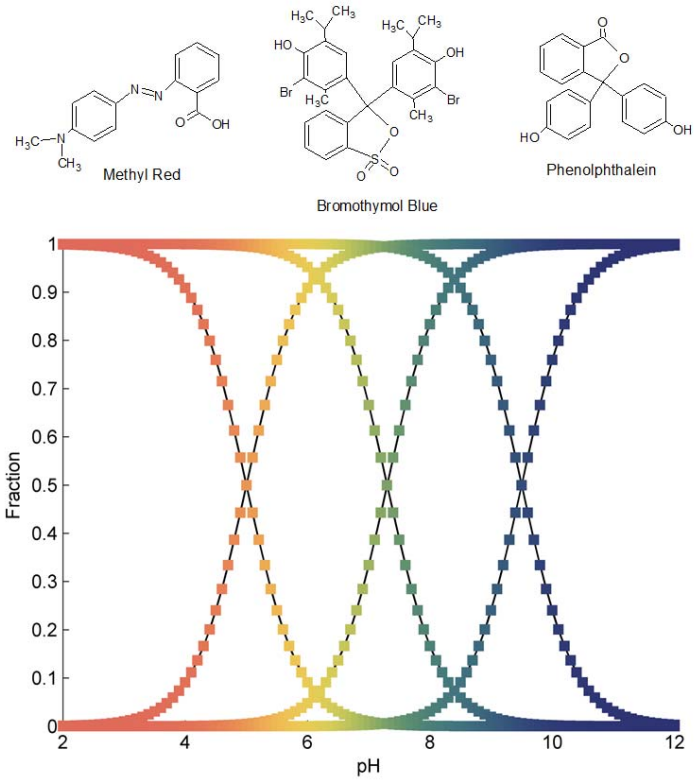

Figure 3: Color Change for Universal Indicator Cocktail, and Associated Indicator Molecules

Naturally, a chemist can visually inspect the color change of a solution with indicator molecules. Because of this, most universal indicators have a test color palette included to visually compare the sample with the colors of known pH values. This is the opposite side of the measuring toolkit: the standard color palette is just a piece of paper, and measurement only takes a glance, but it is impossible to reach more than a 0.5 pH unit level of certainty. The visual inspection method would benefit from increased precision, the spectra from greater speed and ease of use.

Procedure

Fortunately, there exists already a means to determine color quantitatively. A computer screen creates an image by powering different phosphorescent squares; each pixel is made up of one red, one green, and one blue piece. The pixels are small enough that a human eye at a reasonable distance cannot distinguish between them, and so two colors (red and green) appear to blend together to create a different one (yellow). For this reason, the information for a digital image is essentially just the values of each color in each pixel. Images written in this way are said to be in the RGB colorspace – that is, a piece of software that reads the image should look for a number corresponding to red, green, and blue

colors. Digital cameras work in a similar fashion: they create digital images by exposing red, blue, and green light-sensitive cells (Fig. 4).

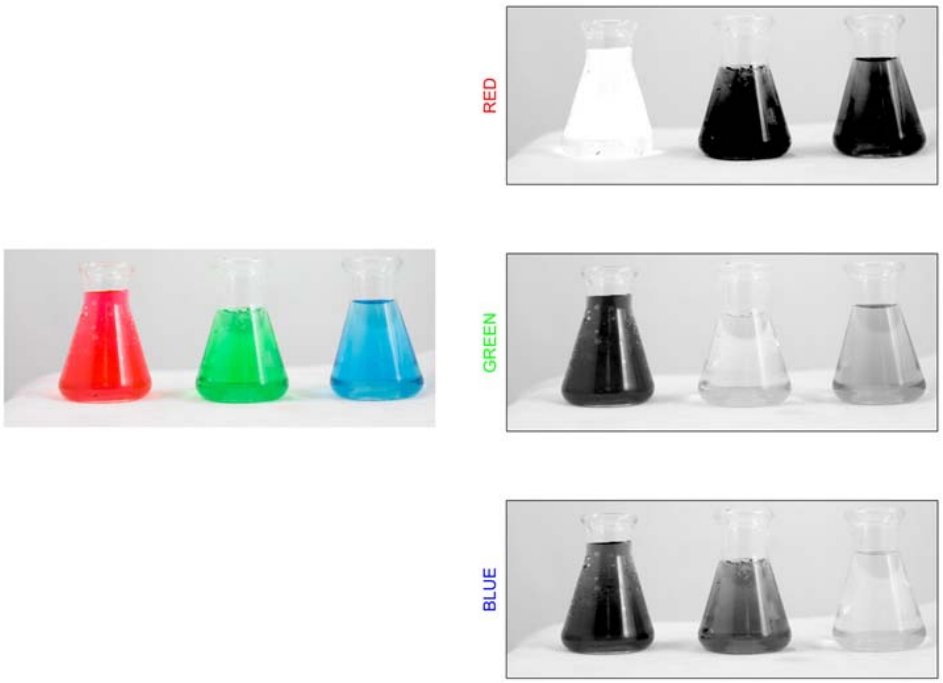

Figure 4: Original Digital Image, and Grayscale of RGB colorchannels

However, while RGB is certainly the most ubiquitous system of digitally representing color, it is not the only one. The HSV colorspace stores the color (Hue), intensity (Saturation), and brightness (Value) as three separate values. While an image will always be displayed in RGB format, it can be converted to HSV with a few simple calculations. For the purposes of this study, only the hue is relevant, and its equation can be seen below (Equation 1). Hue is a qualitative measure of color that wraps from 0 to 1, as shown in the color wheel (Fig. 5). This belies one of the few commercial uses for HSV: it displays color in a way that is intuitive for humans to understand. An origin (a hue of 0) is set arbitrarily on pure red, blue, or green, and the hue of a given pixel is determined by how far it is from the origin.

Equation 1: Hue Calculation from RGB color values

$$H = \begin{cases} \left(\frac{G-B}{max-min} + 0\right)/6; & if\ max = R \\ \left(\frac{B-R}{max-min} + 2\right)/6; & if\ max = G \\ \left(\frac{R-G}{max-min} + 4\right)/6; & if\ max = B \end{cases}$$

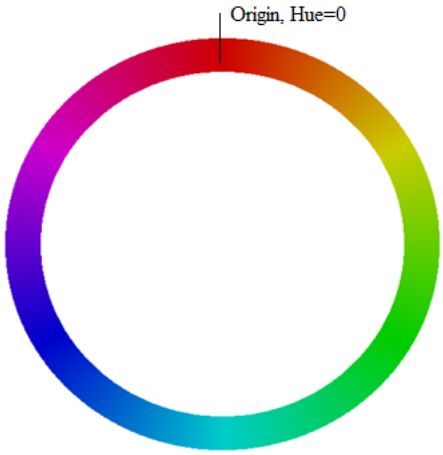

Figure 5: Hue Color Wheel, Origin in Red

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Problematically, looking at an image of a sample with RGB color is fairly complex: the three numbers can vary depending on how bright the picture is. Additionally, the color intensity (how grey or saturated it appears) will also affect RGB color values. However, while certainly the most ubiquitous, this is not the only system of digitally representing color: the HSV colorspace stores those three things – the color (Hue), intensity (Saturation), and brightness (Value) – as three separate numbers. Having one single value for color means the hue is independent of factors like a slightly greyed image. It allows this method to assign a single, quantitative meaning to color.

It is also true that, for an indicator molecule suspended in solution, brightness and intensity change based on how much solution the light passes through (the path length) and how many molecules the light interacts with (the indicator's concentration), but hue is independent of these effects. However, converting an RGB value to HSV must be done for each pixel; any camera made today would have tens of thousands. For this reason, the hue-based method takes the most common hue value. These two

features ensure a single consistent color, regardless of factors like path length, solution concentration, or fringe optical effects from the container.

Test Setup

At present, the experimental setup for the hue-based method uses a digital camera to capture images of a sample of solution and a pH electrode. The general scheme is to titrate a solution with some universal pH indicator. The camera is used to capture images, recording the hue. Measurements from the pH electrode show the change in hue with pH. Because hue is quantitative, a calibration curve (Fig. 6) can be generated between color and pH. If the same indicator solution is then used on a sample of unknown pH, the curve will show quantitatively what the pH is, with higher accuracy than a simple visual determination. This curve is determined by a best fit line, so the result is actually an equation (Eq. 2): hue goes in, pH comes out.

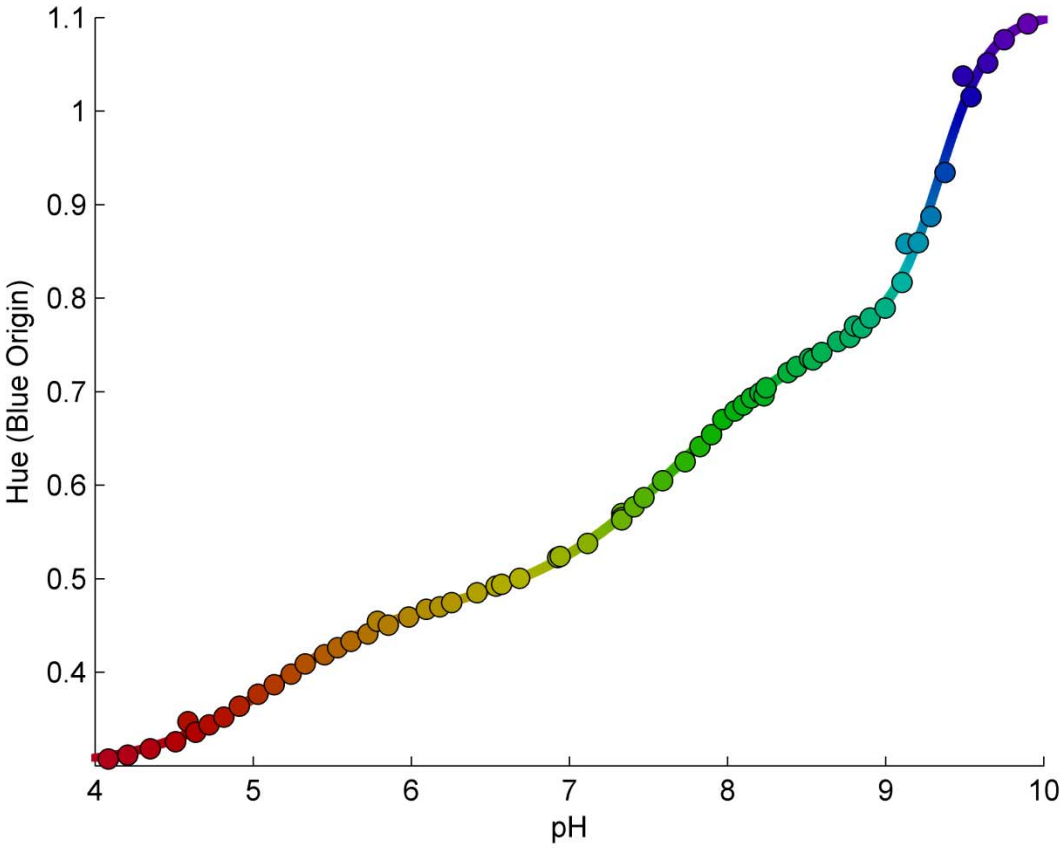

Figure 6: Hue-pH Calibration Curve for Universal Indicator

Equation 2: Calibration Equation for Universal Indicator

$$hue = 1.11 + \frac{-0.17}{1 + e^{\frac{(pH-5.12)}{0.39}}} + \frac{-0.32}{1 + e^{\frac{(pH-7.73)}{0.50}}} + \frac{-0.32}{1 + e^{\frac{(pH-9.35)}{0.16}}}$$

The camera is housed in a light tent surrounded by several fluorescent lamps. This setup prevents the image from being improperly white-balanced: a high amount of red, green, or blue light can cause the color to shift. Normally, cameras have built-in corrections for white-balancing. That is, there is a strong influence on the industry to make sure the color taken by the camera is the one the user sees. However, in developing the standard curve, there is no reason not to take the extra step to make sure the images are as accurate as possible.

Besides the camera and electrode, the experimental setup also includes a large body of Matlab code. Each image has tens of thousands of pixels, each of which requires the hue calculation, so Matlab is a logical choice for this application because it can process all of this data quickly. The Matlab scripts are designed to automatically find images, detect colored regions, and determine the median hue. However, Matlab programs can also connect to physical devices outside of the computer. The equipment also includes an arduino prototyping board. This is connected to the camera, the pH electrode, and four micro-volume pumps. In this way, the software can also control when the camera takes a picture, how fast the titration progresses, and automatically measure the pH. At present, the entire system only needs to be set up with a sample and given specific directions on how to titrate; the rest is automated.

Results

After developing a calibration curve, the hue-based method was tested on a 384 micro wellplate. The container held proteins analyzed for their isoelectric point, a process that requires numerous fractions at varying pH. This is shown in Fig. 6: the color gradient is caused by universal indicator, which shows the pH gradient of each sample. The Matlab program was easily modified to distinguish each well plate, and the hue calculation run. However, checking with the Ross electrode showed the hue calculation was wrong. This problem is not limited to the hue-based method; visual comparison to the standard also yielded the wrong answer.

Testing revealed that a surfactant used with the sample was modifying the color. The chemistry at work here is the same in a kitchen sink: surfactant is basically a fancy word for soap, which allows the protein to interact with water. Unfortunately, it is a necessary part of the process – the protein will not dissolve into solution without it. The color changes but the pH does not, which invalidates both the

calibration curve and the provided comparison standards. With enough detergent, a new curve develops (Fig. 7), and the color stops changing. This point is called the critical micelle concentration, where the surfactant molecules begin to clump together. It seems apparent, then, that the indicator molecules are interacting with the micelles.

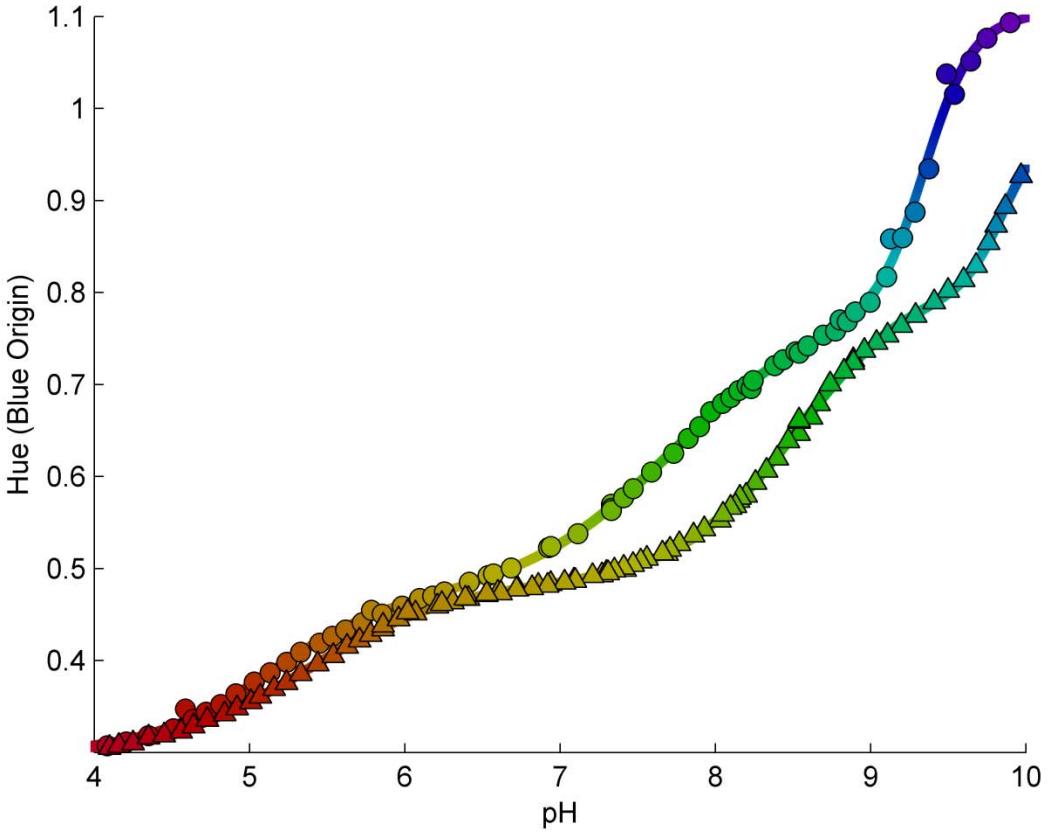

Figure 7: Adjusted Curve in the presence of CHAPS Surfactant

While the new calibration curve (Eq. 3) is just as reliable as the old one, it has a flat spot around a pH of 7. Because of this, anyone using the indicator molecule (with the hue-based method or not) loses sensitivity in this region: any pH between 6.5 and 7.5 will look the same, because the color does not change in that region. A new combination of indicator molecules is being developed, one that has a more consistent color change as pH changes.

Equation 3: Modified Hue Equation for Surfactant Effect

$$hue = 0.98 + \frac{-0.19}{1 + e^{\frac{(pH-5.37)}{0.48}}} + \frac{-0.33}{1 + e^{\frac{(pH-8.52)}{0.38}}} + \frac{-0.17}{1 + e^{\frac{(pH-9.85)}{0.12}}}$$

Discussion

Ultimately, a hue-based method for pH determination is effective and consistent, and offers a dramatic increase in precision over other visual inspections. By using the median hue, a camera can accurately interpret the real color of a solution, regardless of edge effects, variation in indicator concentration, or the depth of the sample volume. The system is not only consistent, but fully automated. Additional issues with white-balancing (ensuring the camera does not over-represent one color channel) are worth consideration, but most digital cameras automatically white-balance images; the solution is built into the technology.

These results also show that the presence of surfactant (as in biological samples) changes the hue but not the pH. This problem exists with any visual method for pH determination, not just the hue-based method. Indeed, making this problem known represents a significant piece of the analytical puzzle for any biochemist. Fortunately, it is also a correctable problem, and a new pH curve has been developed.

Future considerations include a hue-based method for other bio-analytical assays (including a Bradford assay for protein analysis), again making precise quantitative measurement simple and easy. Alternately, if the surfactant issue is indeed caused by micelle formation, use of pH strips (wherein the indicator is immobilized on a membrane) could prove constant regardless of surfactant, and initial tests are promising toward that end. Finally, it is possible the hue-based method could be used on microscope visuals to determine pH at a nano-volume or cellular scale. In principle this is already possible, but as with the surfactant issue there may be unpredictable problems to be addressed before reliable use.