

PH DETERMINATION WITH THE SOLO PLUS



The Hudson Solo Plus Pipettor/Dispenser

Introduction

The accurate determination of pH is important in many chemical and biological applications. This data is often obtained with electrodes; however, this is not a suitable approach for high-throughput microplate-based campaigns, due to the time and accuracy issues introduced by the need to rinse and dry the electrode between readings.

In this AppNote, we describe a simple, automated spectrophotometer-based method for the rapid and accurate measurement of pH in microplates. In this method, we use the Hudson SOLO Plus to prepare the plates for the reader. Note: full automation can be easily achieved by including a PlateCrane to transfer plates between stacks, the SOLO Plus and the reader.

Methodology

The method uses the colorimetric determination of the relative proportion of the neutral and basic form of phenol-based dyes. The example

described here uses bromothymol blue, which is ideally suited for studying pH in the physiological range (7+/-1). Other dyes can be substituted to apply the method to other regions of the pH scale.

Bromothymol blue is a phenol with a relatively weakly bound sulphate group on the alpha carbon at the para substituent (Figure One).

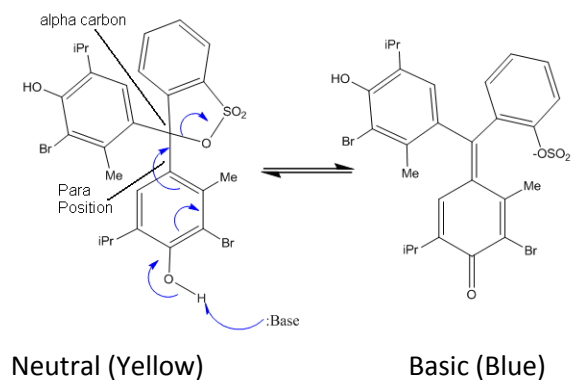


Figure One: Neutral and Basic forms of Bromothymol Blue

Under basic conditions, the phenolic proton is removed and the sulphate group detaches from the alpha carbon as a result of electron resonance through the ring. The neutral, ring-closed form is yellow and absorbs in the visible region between 400-430nm.

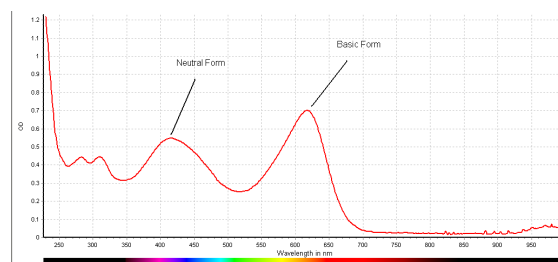


Figure Two: Absorbance spectrum of bromothymol blue at pH = 7

The negatively charged, ring-opened form is blue and absorbs at 620nm. The pKa of bromothymol blue is 7.1, so there is an equal mixture of the two components at a pH of 7.1.

Experimental

The SOLO Plus was equipped with a 200uL 8-channel pipette head and an 8-channel Micro10x in portrait mode at position #2 and primed with bromothymol blue. 100uL disposable tips were placed in position #3 and a 96-well, flat-bottom UV transparent microplate (Costar 3635) was placed in position #2. A reservoir plate containing and standard buffers of pH 4, 6, 6.86, 7, 7.2, 7.4, 7, and 10 was placed in position #4.

20uL of bromothymol blue was added to each well of the microplate using the Micro10x dispenser at 25% maximum speed. Eight replicates of 80uL of standard buffers of pH 4, 6, 6.86, 7, 7.2, 7.4, 7, and 10 were added using the SOLO, changing tips between each buffer. The plate was placed into a BMG SPECTROstar Nano and the full spectrum (230-1000nm) was read after 15 seconds of double orbital stirring at 500rpm. The raw data was analyzed with BMG's Mars software.

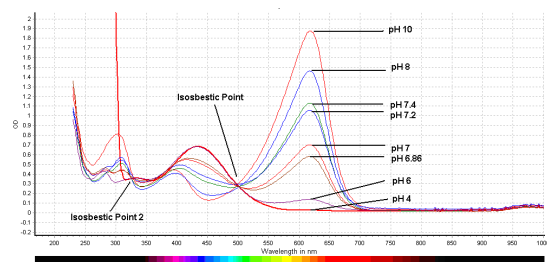


Figure Three: Overlap of absorption spectra of bromothymol blue in 8 different standard buffer solutions (pH 4, 6, 6.86, 7, 7.2, 7.4, 8, 10).

We used a BMG LabTech spectraSTAR reader to obtain the absorbance spectra. This instrument allows one to obtain the entire spectrum for each well in less than 2 minutes. Alternatively, a standard monochromometer or

filter-based instrument can be used. In either case, collect data for the absorbance at 630nm and 750nm. Subtract the 750nm value from the 630nm to maintain a common baseline and determine the statistics for each column.

Results

Figure Two shows the absorption curve generated with the pH 7 buffer in which the concentration of the two forms of the dye are roughly the same.

The variation of the absorption curves with pH is best seen in figure Three in which the spectra determined at each pH have been overlapped. The overlap shows the isosbestic points at 499nm and 326nm

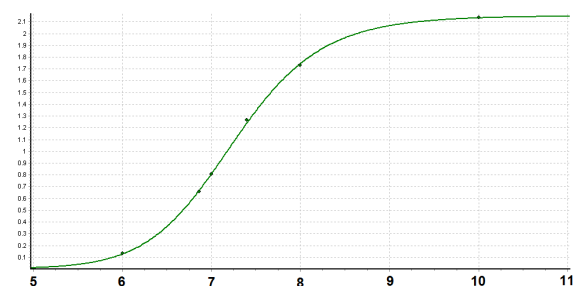


Figure Four: Plot of pH dependence of the absorbance of the basic form of bromothymol blue (620nm – the 750nm baseline).

Figure Four shows the relationship between absorbance maxima versus pH. Mars fitted the data to a 4 point curve with a regression coefficient of 0.9997.

Conclusion

This method represents a simple, fast and accurate method of determining the pH for a large number of samples in microplates without the delays and inaccuracies inherent in electrode-based measurements.

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