MEASURING pH USING PHOTOPETTE® CELL

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- *Photopette can be used for photometric pH measurements of high salinity samples such as seawater and culture media*

OBJECTIVE

This application note is a simple guide to determine the pH of *Spirulina Platensis* culture medium by measuring absorbance at 680 nm using Photopette®. This method can be easily adopted by industries culturing *Spirulina Platensis.*

INTRODUCTION

Growth of the cyanobacteria *Spirulina (Arthrospira) Platensis* occurs at an optimum pH of 9 – 10 [1]. Due to the very high salinity of the culture medium, conventional pH meters do not provide accurate results. During the cultivation process, the production of primary and secondary metabolites in the culture give rise to a pH change in the medium and drive the process to a sub-optimal pH range. In order to achieve an optimal growth, the pH of the cultivation medium needs to be frequently monitored and adjusted to fall within the optimum pH range. An ideal pH indicator for monitoring the pH of the medium must have a response in the range of pH 9 – 11. Thymolphthalein has been chosen as the pH indicator for the current experiment because its response range lies between pH 9.3 to 10.5. The colour transition occurs from colourless (below pH 9.3) to blue (above 10.5). The molar extinction co-efficient for the blue thymolphthalein dianion is 38,000 M^{-1} cm^{-1} at 595 nm [2].

MATERIALS AND APPARATUS

Instrument:

- Photopette® Cell with 680 nm wavelength

Reagents:

- Sodium bicarbonate ($NaHCO_3$) (Sigma Aldrich #S5761)
- Sodium carbonate (Na_2CO_3) (Sigma Aldrich #791768)
- Thymolphthalein indicator (Sigma Aldrich, #114553)
- Ethanol (Sigma Aldrich, #L7022)

METHOD

EXPERIMENTAL PROCEDURE

Sample preparation for standard curve: 0.1 M solutions of $NaHCO_3$ (2.1 g in 250 mL DI water) and Na_2CO_3 (2.65 g in 250 mL DI water) were mixed in the following proportion (see Table 1) to prepare 25 mM of carbonate buffer solutions. 1% (w/v) solution of thymolphthalein was prepared in ethanol, and 20 µL thymolphtalein solution was added into 1 mL of the prepared standard buffer. A blank was prepared using DI water. The solution was mixed using a vortex mixer and absorbance reading was measured at 680 nm using Photopette® [3].

pH	Volume (mL) of 0.1 M $NaHCO_3$	Volume (mL) of 0.1 M Na_2CO_3	Volume (mL) of DI water
9.22	22.50	2.50	75
9.62	18.12	6.88	75
9.82	15.94	9.06	75
10.14	13.75	11.25	75
10.39	9.37	15.63	75
10.57	7.19	17.81	75
10.69	5.00	20.00	75

Table 1: Proportion of $NaHCO_3$ and Na_2CO_3 required for preparation of 25 mM carbonate buffer in the pH range of 9.22 to 10.69.

Protocol: Filter the culture medium using 0.2 µm syringe filter. Pipette 1 mL of the filtered medium into 1.5 mL tube. Add 20 µL thymolphthalein solution (1% w/v in ethanol) and mix it using a vortex mixer. Prepare a 'blank' using culture medium

alone, without adding the indicator dye. The sample is now ready for measurement.

Measurement: Turn on the Photopette® Cell device and connect to the Photopette iOS/Android app. Refer to the Photopette® user manual for operating and safety precautions [2]. Select '680 nm' as the wavelength. Select dataset and set additional settings (if needed) before selecting 'Start Measurement'. Please follow the video tutorials available at www.tipbiosystems.com to get familiar with the measurement process.

Place a CuveTip® firmly onto the Photopette® device probe and insert into the blank sample to perform auto-zero. Ensure that there is no air-bubble trapped in the CuveTip® cavity. Presence of air bubbles disrupt the optical path and cause errors. A guide to use the CuveTip® correct is available as download [3].

Use the same CuveTip® to measure the standard pH solutions from low to high concentration of hydroxide. Ensure that there is no liquid transferred between samples by contacting the CuveTip® with a tissue paper and remove any liquid by capillary action. Repeat the procedure for the samples with unknown pH.

Determine the pH of S. Platensis medium

Measure the absorbance of the mixture of 1 mL filtered medium and pH indicator. S. *Platensis* culture medium does not require further dilution. Sample preparation and measurements should be done as duplet or triplet. The concentration of hydroxide ions [OH^-] is calculated by substituting the absorbance value obtained into the equation of the standard curve.

RESULTS AND DISCUSSION

STANDARD CURVE

Colour transition of thymolphthalein indicator dye occurs from pH 9.3 (colourless state) to pH 10.5 (deep blue colour) as shown in Figure 1. The standard curve (absorbance vs. [OH-] and absorbance vs. pH) at 680 nm is shown in Figure 2. Absorbance is linearly proportional to the concentration of hydroxide ions present in the sample with a slope of 3381 AU/M. The R^2 value for the best linear fit is 0.989 (Figure 2A). Using the standard curve generated, the pH of the filtered Spirulina medium was measured to be 10.36 with ~2% error; the same medium was measured to be 10.14 using pH meter.

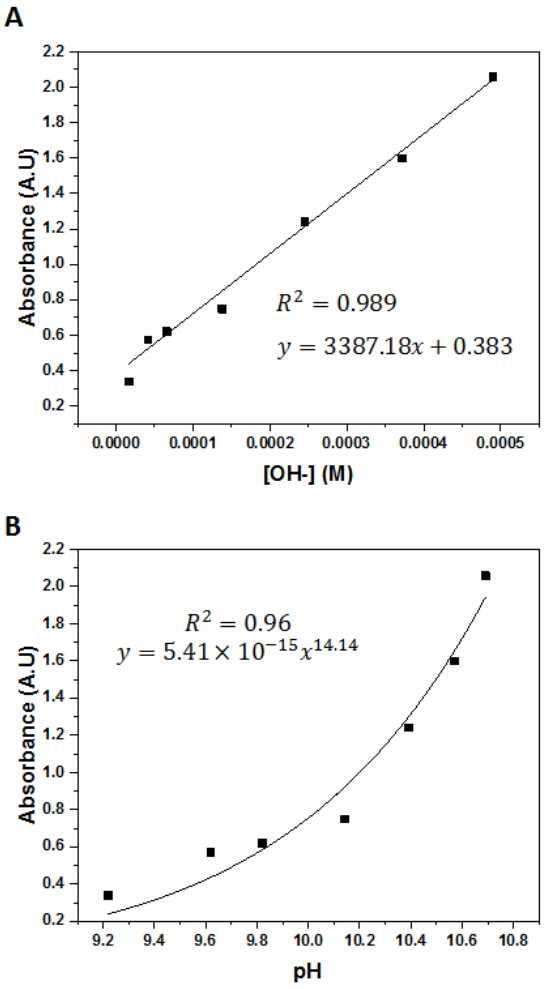

Figure 1: *A)* Standard curve *(*Absorbance vs. [OH-]*)* of mixture of thymolphthalein and carbonate buffer*, B) Absorbance vs. pH of mixture of thymolphthalein and carbonate buffer.*

Photopette® users may download a pre-configured worksheet for pH measurement from our online resource section. The worksheet is compatible with Microsoft Excel® and similar worksheet software and will aid users in generating the standard curve. It is advisable to prepare a complete standard curve with at least 5 data points (hydroxide ion concentrations) and a blank for the first measurement to confirm linearity. For follow up measurements, it is advised to

run one or two pH standards together with the culture medium sample.

EXPERIMENTAL PAPRAMETERS

Linear range

The calibration curve of Figure 2 shows a linear range in the pH range of 9.22 to 10.8.

pH OF CULTURE SAMPLES

Triplicate of *S.Platensis* culture samples were measured following the procedure outlined in the 'protocol' section. An average reading of absorbance of 1.162 was obtained. The linear regression of the pH standards of Figure 2A is:

$$y = 3387.18x + 0.383$$

Rearrangement to the lactate concentration "x" results in:

$$x = \frac{y - 0.383}{3387.18}$$

Inserting the absorbance of the unknown sample results in:

$$x = \frac{1.162 - 0.383}{3387.18}$$

$$x = 2.3 \times 10^{-4}\,\text{M}$$

$$[OH^-] = 2.3 \times 10^{-4}\,\text{M}$$

$$\text{pOH} = 3.63$$

$$\text{pH} = 14 - \text{pOH}$$

$$\text{pH} = 10.36$$

EXPERIMENTAL VARIATIONS

The same measurement procedure can be used to determine hydroxide ion concentrations in other samples such as sea water, brackich water, colorless beverages, food, biological samples including serum, plasma and fermentation media.

LIMITATIONS

The pH range that can be measured using Thymolphthalein dye is in the range of 9.22 to 10.8. The limitation in the range is the result of the operating range of Thymolphthalein indicator. For pH measurement outside the range, new methods need to be established using different pH indicator dyes.

SUMMARY

This application note guides a user in determining pH in the range of 9.22 to 10.8 in various samples by measuring the absorbance at 680 nm. The experiment is low cost and easy to conduct. The method can be easily adapted by industries culturing *S. Platensis*, analytical laboratories, or for school experiments.

REFERENCES

[1] Newsted, John L. "Effect of light, temperature, and pH on the accumulation of phenol by Selenastrum capricornutum, a green alga." *Ecotoxicology and environmental safety* 59, no. 2 (2004): 237-243.

[2] Sigma-Aldrich, Product description of Thymolphthalein #114553

[3] Tip Biosystems Pte Ltd, Technical Note "How to use Photopette's CuveTip correctly"