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Colorimetry-based quantification of protein droplets using "ImageJ"

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Abstract

The objective of this study is to analyze protein concentration on a colorimetric basis by utilizing the openly accessible 'ImageJ' software. We herein report the successful measurement of protein concentration on a parafilm surface, following the application of Bradford reagent and subsequent photography with a smartphone, followed by analysis using the ImageJ software. Ovalbumin (OVA) and bovine serum albumin (BSA) were dissolved in phosphate buffered saline (PBS) and used as standards. The results demonstrated a linear correlation between integrated density and the actual weight of the standard, with R^2 values of 0.9908 for OVA samples in the range of 0-1.5 mg/ml and 0.9781 for BSA in the range of 0.25-1.5 mg/ml. The straightforward nature of the experiments and analyses makes the study we present here an invaluable resource for researchers and students who are constrained by equipment limitations, such as the lack of access to spectrophotometers.

Keywords: Bovine serum albumin; Ovalbumin; Protein quantification; Smartphone

1. Introduction

In biochemistry, biology, or molecular biology laboratories, protein quantification is a routine and essential task. It is crucial at every step of processes like isolating and purifying proteins from animals, plants, or microorganisms, whether they are naturally occurring or genetically modified (1). For this purpose, several methods are commonly employed in laboratories, including UV absorbance, the Bradford assay (2), the bicinchoninic acid (BCA) assay (3), and the Lowry assay (4). Each method involves the use of a spectrophotometer, but they differ in wavelengths and reagents. For instance, the UV absorbance method detects aromatic amino acids like tryptophan and tyrosine at 280 nm. The Bradford assay uses Coomassie brilliant blue dye, which binds to proteins, causing a shift in absorbance from 465 nm to 595 nm. The BCA assay relies on the reaction between BCA and copper ions (Cu+) generated when proteins reduce Cu^{2+}, producing a color change measurable at 562 nm. In contrast, the Lowry assay combines the Biuret reaction (protein-copper complex) with Folin-Ciocalteu reagent, yielding a color change detected at 750 nm (5) and (1).

Estimating protein concentration through color changes is especially useful when OD 280 nm absorbance cannot be used. For faster results without reagent mixing, UV analysis at OD 280 is a practical alternative. However, all these methods rely on a spectrophotometer, typically a UV-Vis type. In cases where such equipment is unavailable, alternative approaches have been explored. The widespread availability and affordability of smartphones have helped address this limitation. Colorimetric assays are well-suited for smartphone-based analysis. Gee et al. (6) demonstrated how smartphone-based colorimetry can effectively measure protein concentrations using two popular biochemical methods, the Bradford assay and the biuret assay.

Quantification in these assays is achieved by measuring visible light absorption and applying Beer's Law to convert absorbance into concentration using a standard calibration curve. The Bradford and biuret tests are two common

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colorimetric methods. By using known standards within the linear range of Beer's law, a calibration curve can be created to estimate the concentration of unknown samples (1). Common standard proteins include bovine serum albumin (BSA) and ovalbumin (OVA).

Researchers can perform quantitative analysis of absorbance values using a spectrophotometer, with immediate results available if the device is equipped with appropriate software. Unlike spectrophotometer-based methods, dot blots on membranes typically require scanning before quantification with software like ImageJ. Aznar et al. (7) used ImageJ to measure Vibrio cholerae O1 lipopolysaccharide concentration via an immunodot blot assay. Similarly, Kinenda et al. (8) successfully analyzed immunodot blot results on membranes using ImageJ 1.54i.

In this study, we present a colorimetric-based approach for quantifying protein spotted on parafilm sheets using the Bradford assay, followed by analysis using the ImageJ software (http://imagej.org).

2. Material and methods

In this study, Bradford reagent (Sigma) was used as a dye, with ovalbumin and bovine serum albumin (Sigma) serving as the protein standards. The procedures were modified from Kielkopf et al (9) .The proteins were weighed and dissolved in phosphate-buffered saline (PBS) at pH 7.4 to prepare concentrations ranging from 0 to 1.50 mg/mL in increments of 0.25 mg/ml.

A 5 µL aliquot of each protein standard was dispensed onto a flat sheet of Parafilm (Parafilm-M®), followed by the addition of 20 µL of Bradford reagent. The mixtures were incubated at room temperature, as recommended by Bradford (2). Images of the resulting color changes were captured using a Samsung smartphone. The experiment was conducted in duplicate, and the images were analyzed using ImageJ, following the software provider's guidelines (http://imagej.org).

3. Results and discussion

The main objective of this study is to help researchers, laboratory technicians or students to perform protein estimation based on the Bradford reagent staining method. The study employed bovine serum albumin (BSA) and ovalbumin (OVA) as experimental materials, which are commonly utilized in biological laboratories. Figure 1 depicts photographic images of parafilmM sheets of varying concentrations of BSA (Figure 1A) and OVA (Figure 1B) following reaction with Bradford dye.

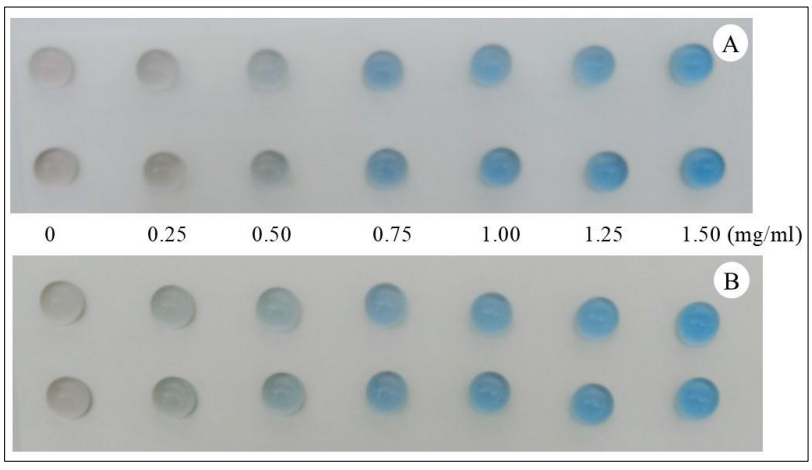

Figure 1 Representation of smartphone images of BSA (A) and OVA (B) spots with a concentration range of 0 to 1.5 mg/ml after the addition of Bradford reagent dripped on the parafilm surface

Qualitatively, it can be clearly observed that there was a change in color intensity of the Bradford reagent. The color change occurred gradually starting from droplets that do not contain protein to those with higher protein content. This is a typical of the Bradford assay using Coomassie brilliant blue dye, which binds to proteins (10), (1). The color change is due to the chemical reaction between Coomassie blue G-250 dye and protein, primarily to basic (especially arginine) and aromatic amino acid residues (11).

The main purpose of measuring protein concentration in the laboratory is to obtain quantitative values for use in further stages. Therefore, qualitative observations alone are not sufficient. In general, Bradford reaction results are read using a spectrophotometer at a wavelength of 465 nm to 595 nm. Previous research has demonstrated that the spots depicted in Figure 1 can be quantified using software such as ImageJ (7) and (8). This software, when employed in conjunction with a smartphone, provides an alternative solution when laboratory faces the obstacles facilities are lacking in the necessary spectrophotometer equipment.

The procedure for utilizing the ImageJ software is straightforward, allowing for its implementation by individuals without specialized training. In accordance with the operational instructions of ImageJ, the intensity quantification of each spot is expressed as integrated density, which is positioned on the Y-axis, while the actual protein concentration (in mg/ml) is positioned on the X-axis. The results of the quantitative analysis utilizing ImageJ software are presented in Figures 2 and 3 for BSA and OVA standards, respectively. The analysis of BSA and OVA standards dripped on parafilm produced linear curves with R2 = 0.9781 and R2 = 0.9908, respectively.

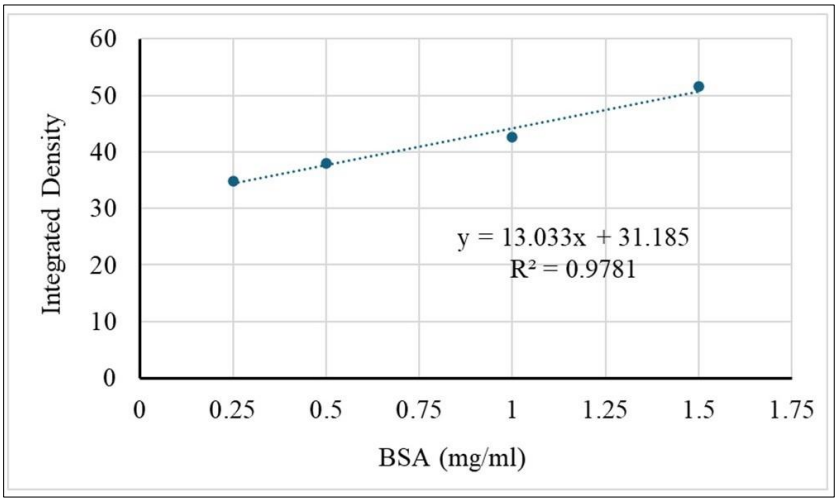

Figure 2 The calibration curve of BSA standard protein, treated with Bradford reagent and analyzed using ImageJ, represents the average values from three replicates.

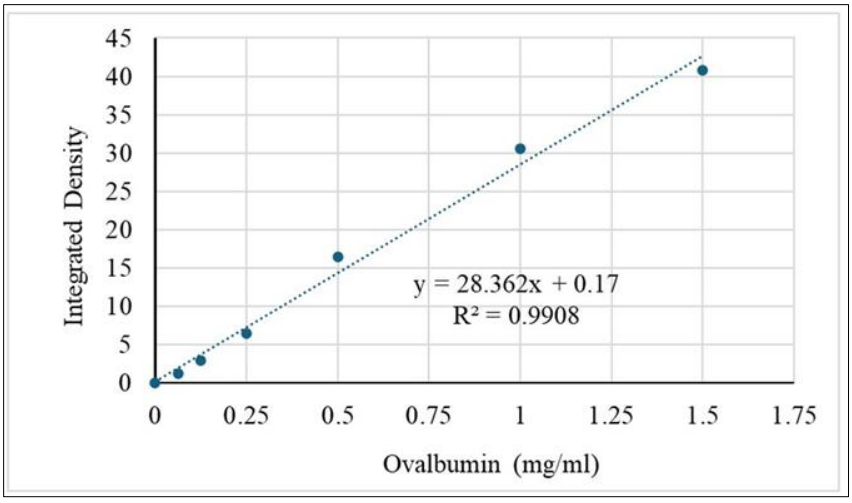

Figure 3 The calibration curve of OVA standard protein, treated with Bradford reagent and analyzed using ImageJ, represents the average values from three replicates

4. Conclusion

Overall, the approach of measuring protein concentration colorimetrically by utilizing the openly accessible 'ImageJ' software can be adapted as an alternative to overcome the obstacle of spectrophotometer availability. Nevertheless, further research is still needed using protein samples with unknown concentrations.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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