Research article

Sensitive and rapid Bradford and BCA protein assay by a common desktop scanner

Shahila Parween[1], Pradip Nahar [2]

[1]MNR Foundation for Research & Innovations (MNR-FRI), MNR Medical College & Hospital, MNR Nagar, Fasalwadi, Sangareddy, 502 294, Telangana, India

[2](Superannuated as Chief Scientist from CSIR-IGIB) CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi, 110 007, India

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Corresponding author: **Shahila Parween.** Email: Shahila.fri@mnrindia.org

ABSTRACT

Introduction and Aim: The conventional method of protein quantification involves the need for a costly spectrophotometer which is a bulky and expensive instrument. Also, it requires a considerable quantity of protein which is often valuable or not affordable. In this communication, we report a sensitive image-based protein assay method overcoming the challenges faced by the conventional method. BCA and Bradford protein assay in a miniaturized form is carried out on a polypropylene micro-test plate (PPμTP) using only 1 μl of the precious protein solution in the nanogram range without the need for expensive equipment for quantification.

Materials and Methods: In this procedure, after the assay reaction, the assay plate with a color solution is turned upside down for capturing images in a desktop scanner. The image is then quantified digitally by a color space using freely available Adobe Photoshop and Macbeth color calculator software.

Results: Standard graphs made by the present image–based method agreeably correlate with the absorbance–based method carried out in a microtiter plate with a Pearson coefficient of 0.995178 and 0.981006 for BCA and Bradford assay respectively. A test protein quantified by this method shows an accuracy of 97%. Further, we have reduced the protein assay time to 75 s only by performing the assay on PPμTP by microwave irradiation.

Conclusion: Image-based protein assay is also performed in an ultra-miniature assay plate (UAP) which requires only 1 μl of assay solution, reducing the detection limit further to the 10 femtogram/test zone. In short, image–based protein assay on PPμTP and UAP platforms could be an outstanding alternative either to spectrophotometric or paper–based protein assay.

Keywords: Polypropylene micro-test plate (PPμTP); desktop scanner; image analysis; protein assay; enzyme.

INTRODUCTION

Traditionally, protein is quantified colorimetrically by different methods (1) including Lowry (2), bicinchoninic acid (BCA) (3), and Bradford (4). All these methods need a spectrophotometer which in itself is a bulky and expensive instrument. Also, it requires a considerable quantity of protein which is often valuable or not affordable. Therefore, it is necessary to have a device or assay system that is low-cost, sensitive, instrument-free, and most importantly, requires a small amount of protein sample. The image-based biochemical assay method could be a possibly useful solution for such an assay technique. Prerequisites for an image–based system include (i) a mini reaction analysis device, (ii) an image-acquiring instrument, and finally (iii) an image analytical tool. In the past, paper (such as Whatman paper) discs were used as a reaction device for several chemical assays such as spot test assays (5), or biochemical assays such as paper discs ELISA assays (6,7). Mostly, these assays were qualitative. Recently, some researchers are trying to make paper-based assays quantitative by capturing images of the assay solution. Most of the image-based colorimetric assays on paper were used for glucose estimation, pH determination, lipid estimation, blood grouping, or different components measurement in a sample (7-16). Image-based ELISA is reported on a polypropylene micro-test plate as an excellent substitute for conventional ELISA in a microtiter plate (17).

In the present work, image–based protein quantification by BCA and Bradford methods on polypropylene micro test plate (PPμTP) using color saturation has been carried out (Fig. 1). The results of the present method are further compared with spectrophotometric quantification. Further, we have conducted a protein assay on paper discs for comparison.

MATERIALS AND METHODS

Reagents and chemicals

Bovine serum albumin (BSA) and Chicken egg albumin were obtained from Sigma - Aldrich (USA). Protein estimation kits involving bicinchoninic acid (BCA) and Bradford procedures were purchased from G-Biosciences (USA, Cat. No.786-570) and Bio-Rad, USA respectively. All buffer solutions were freshly prepared in triple DW. To prepare phosphate-buffered saline (PBS) 0.85% NaCl was mixed with 0.01M phosphate buffer (pH 7.2). Microwave-mediated protein estimation was done in a domestic microwave oven functioning at a frequency of 2450 MHz with a

power of 700 watts. Paper discs were made from Whatman paper (Sigma - Aldrich, USA) using a paper punch. The experiments were performed in triplicates.

Procedure for making PPµTP and paper discs

PPµTP was prepared from polypropylene sheets having 0.8 mm thickness which was locally purchased as in our previous work (17). Briefly, the sheet was cut into a strip of dimensions of 9 cm X 3 cm. Onto the strip, an array of zones was fabricated. The test zones of the PPµTP have a diameter of 3.5 mm and a depth of around 1 mm. Each test zone required only 8- 10 µl of the sample, nonetheless, a test zone of reduced volume can be made easily like an ultra-miniaturized assay plate (1µl volume plate) (18). We may also add that the present method is based on the image of the solution which is the intensity of the color of the solution. In other words, for a defined color solution, the image (color) will be the same irrespective of little variation in the volume of the solution. The minimum requirement is that the solution should cover the whole surface of the cavity. To ensure uniformity of results we have taken the same amount of reagents for each cavity. Paper discs were made from Whatman paper with the help of a paper punch. The disc's diameter was similar to the diameter of the PPµTP cavity.

Spectrophotometric BSA quantitation by the BCA method

For spectrophotometric quantification of BSA by the BCA method, different volumes (0.5, 1, 1.5, 2, 2.5 and 3) µl BSA from a stock solution of 2 mg /ml were loaded into the wells of a microtiter plate. To each of these wells, distilled water was added to make the final volume in each well to 10 µl. Bicinchoninic acid and copper sulfate solutions from the BCA assay kit (G Biosciences, Cat. No.786-570) were mixed in a ratio of 50:1 as per the supplier's protocol. From this, 100 µl was added to each well having protein solution. The assay plate was then incubated at 37°C in a hot air oven for 30 minutes. The experiment was performed in triplicates. Absorbance from the microtiter plate was recorded at 570 nm in a microtiter plate reader.

Spectrophotometric BSA quantitation by the Bradford method

BSA estimation by the Bradford method was done by taking different volumes (0.5, 1, 1.5, 2, 2.5, and 3) µl of BSA from a standard solution of 2 mg /ml into the test wells of a microtiter plate. To each of these wells, distilled water was added to make the final volume in each well to 10 microliters. Bradford reagent (5X) was diluted to 1X using distilled water. Then 200 µl of Bradford reagent (1X dilution) was loaded into each well. The polystyrene microtiter plate was kept at room temperature for 5 minutes. Absorbance from the microtiter plate was recorded at 595 nm in a microtiter plate reader (Bio-Rad iMark™ Microplate Reader, USA).

Image-based BSA quantitation by BCA and Bradford methods

The image-based analysis was done using the same-colored assay solution as obtained from the above sections, spectrophotometric BSA quantitation by the BCA and Bradford methods. After taking the absorbance from the above experiments, colored solutions from microtiter plates were transferred to PPµTPs (10 µl per cavity) and paper discs (6 µl per disc) for image-based assays. Paper discs were arranged on a transparency sheet in the same layout as PPµTP. Images of PPµTP with assay solution were obtained after scanning on a desktop scanner, HP Photosmart C6388. To do so, we overturned the plate and then scanned it; owing to surface tension, the solution remained in the cavities without any spilling. However, paper discs images were scanned after drying them. Adobe Photoshop was then used to quantify the scanned images in terms of color saturation (17). A graph was drawn to correlate color saturation and absorbance pertaining to BSA concentrations. We have chosen saturation as a color space because it gives a good correlation with absorbance as reported in our previous paper (19).

Quantification of a test protein on a PPµTP and paper disc plate by an image-based method

BSA standards by BCA and Bradford procedures were made directly on PPµTPs and paper discs (plates) using 1µl of protein solution for each cavity from stock solution (10-60) µg/ ml followed by the addition of 9 µl of BCA or Bradford reagent. The assay plates were then incubated at 37°C, for BCA: 30 minutes and Bradford assay: 5 min. After the assay, color saturation was calculated from their images as mentioned in the above section. Now, different amounts of chicken egg albumin: CEA (25 and 40) µg/ml were taken as a test sample and loaded into the cavities of PPµTPs and on discs of paper plates. After assay by both the protein assay methods, color - saturation was calculated from their images. Putting these values on the standard graphs, the amount of CEA was estimated.

Ultra–fast protein estimation

BSA standard by BCA was also made by microwave irradiation instead of thermal incubation in a domestic microwave oven with a power of 700 watts operating at a frequency of 2450 MHz. PPµTP together with protein solutions and reagents was kept inside the microwave oven for 75 seconds. Microwave irradiation time was optimized before the experiment. Now, a known amount of CEA (25 µg/ml) was taken as a test sample and loaded into the cavities of a fresh PPµTP. After adding BCA reagents PPµTP was exposed to microwave irradiation for 75 s. The plates were then scanned, and color saturation was then calculated as mentioned above.

Protein quantification on an ultra-miniaturized assay plate (UAP)

BSA standards by BCA procedure were made on UAP in the first seven rows in triplicates by loading 1µl of BSA solution for each cavity from stock solution (10000 - 0.01) ng/ ml. In the next two rows, the protein to be analyzed, CEA, was taken in two different concentrations (100 and 10) ng/ml. Subsequently, 1 µl of BCA protein assay solution was loaded into each cavity. The assay plate was then incubated for 6 minutes at 37°C. The plate was then scanned with assay solution using a desktop scanner and then quantified as saturation percentage. Putting the image intensity values of the analyte on the standard graphs the amounts of analyte protein were estimated.

RESULTS

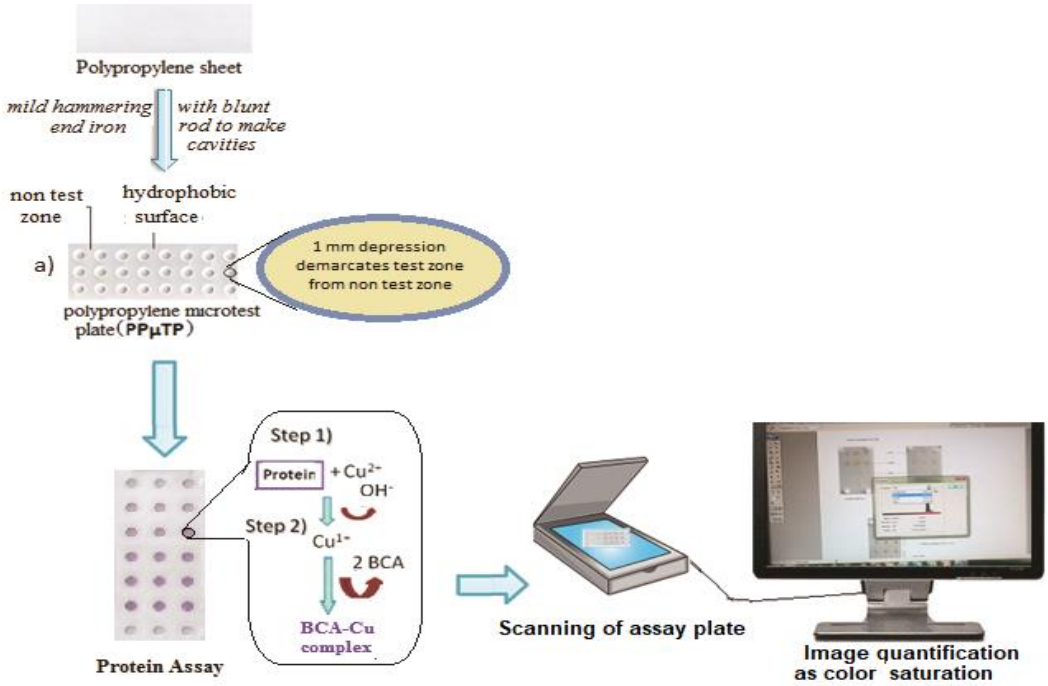

Fig. 1: Overview of an image–based protein assay.

For protein estimation, BSA was used conventionally to make protein standard graphs by BCA and Bradford procedures. First, different concentrations of BSA spectrophotometrically on microtiter plates were estimated. As lower amounts of BSA failed to give detectable absorbance, we have made a standard graph using 1-6 µg of BSA. Color-saturation-based assays were done on PPµTPs and paper discs with the same assay solutions.

From fig. 2 a strong correlation between color saturation and absorbance with respect to BSA concentrations was observed. Fig. 3. represents the results obtained by quantifying a test protein CEA through image–based methods on PPµTP. A good estimation of test protein could be observed through the BSA standards curves for both BCA and Bradford procedures Also, microwave-mediated test protein evaluation by the BCA method. Fig. 4 showed an excellent result of protein estimation in a miniature plate utilizing only 1µl of the assay protein.

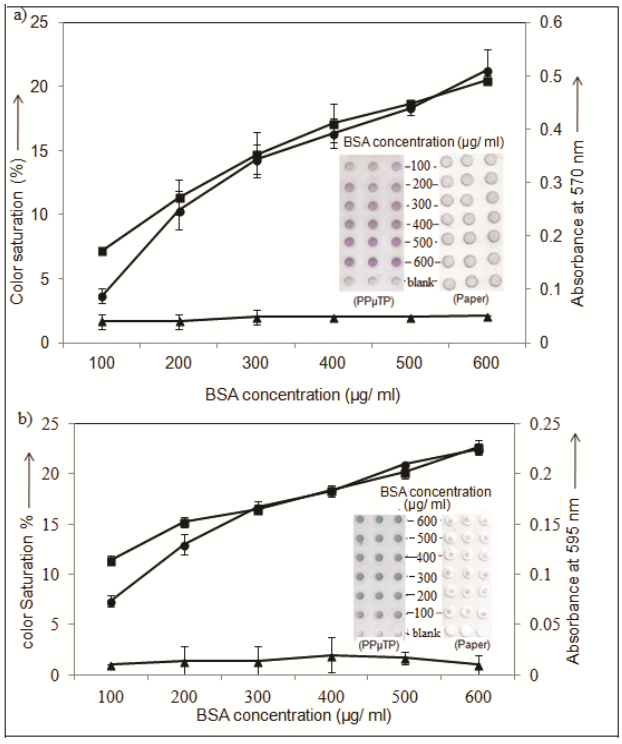

Fig. 2: Absorbance and color-saturation based BSA quantitation by (a) BCA and (b) Bradford procedures. Absorbance values (■) were recorded in a spectrophotometer. Color- saturation based quantitation was done on PPµTPs (●) and paper discs (▲) from the scanned images (inset) of the same assay solutions used for absorbance reading.

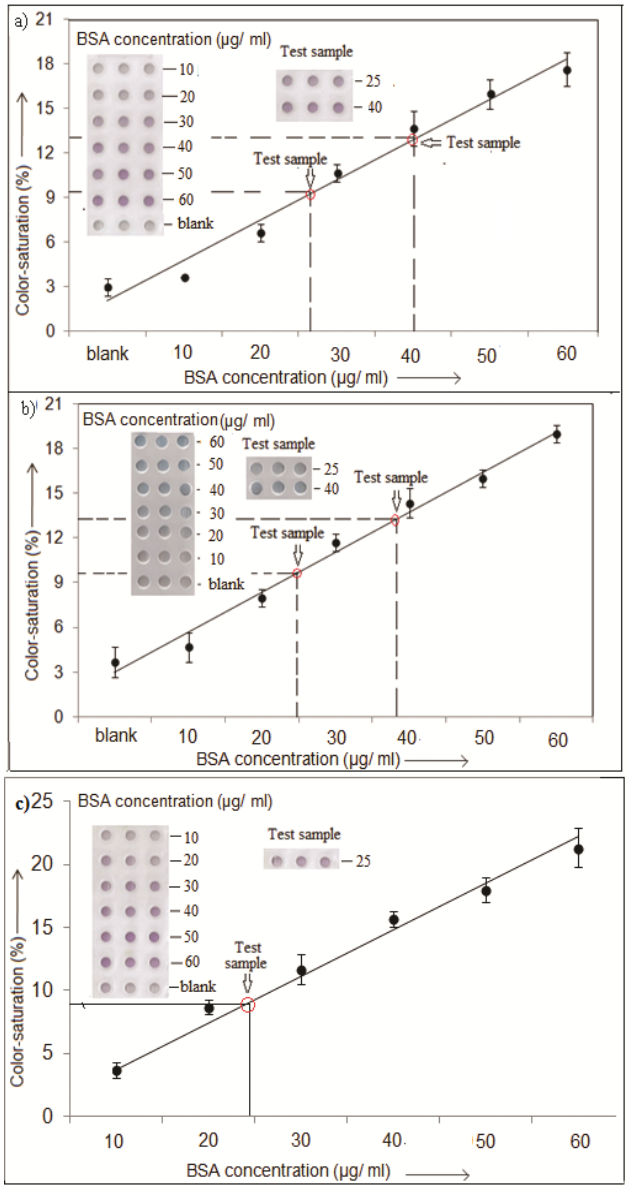

Fig. 3: Quantification of a test protein (chicken egg albumin) from BSA standards made by (a) BCA (b) Bradford procedures on PPµTP via color-saturation and c) by microwave-mediated protein estimation on PPµTP by BCA method.

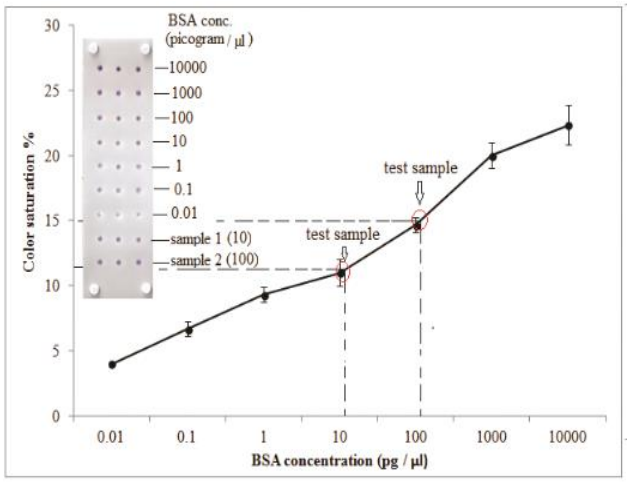

Fig. 4: BSA standard graph is made using different concentrations of BSA and quantifying them by color saturation values on a miniature assay plate. Quantification of the test protein is determined by putting the color saturation values on the BSA standard graph after its assay.

DISCUSSION

An image–based assay method is budding as a potential tool for affordable diagnostics (8). From the graph of fig. 2 strong correlation between color-saturation and absorbance pertaining to BSA concentrations with a Pearson correlation coefficient of 0.995178 and 0.981006 for BCA and Bradford assay respectively. We have conducted the experiments on paper discs because most of the image - based assays are reported on paper discs or strips. However, no standard graph could be made from the results on paper discs as paper itself reacts with both the protein assay reagents, giving erroneous results. Also, the color disappears from the paper discs as soon as it becomes dry as these reactions are based on ionic interactions in an aqueous solution.

To find out the minimum concentration of protein that can be assayed on PPµTP, we have made BSA standards for both methods. From this standard (Fig. 3), we have quantified different concentrations (25 and 40) µg/ ml of chicken egg albumin (CEA) on PPµTP. The BCA method on PPµTP shows 26 and 40 µg/ ml of CEA (Fig. 3a) whereas the Bradford method shows 24.5 and 38 µg/ ml of CEA (Fig. 3b). However, a conventional microtiter plate failed to detect such a low concentration of a protein because of the higher volume of solution required in a microtiter plate for recording absorbance which is made by diluting the solution; hence, significantly decreasing the absorbance value. Finally, we have decreased the BCA protein estimation time on PPµTP to 75 s from 30 minutes by performing the incubation procedure by microwave irradiation (Fig. 3c) which was performed in a domestic microwave oven (20). The efficacy of the microwave estimation procedure is further validated by estimating the known amount of analyte (CEA). This shows 24 µg/ ml of chicken egg albumin (the actual amount 25 µg/ ml).

Image – based protein assay was further carried out on a 1 µl volume plate. Different concentrations of BSA on UAP were assayed to make a standard graph (Fig. 4). Chicken egg albumin was taken in two different concentrations as an analyte protein and assayed. Values of analyte protein in different concentrations were put on the standard graph and calculated the amount of analyte protein. Fig. 4 shows (11 and 110) ng/ ml of protein which is in close agreement with the actual amount taken (10 and 100) ng/ ml. The method is highly sensitive and could be an important tool to quantify proteins that are precious or not available in plenty.

CONCLUSION

As image-based protein quantification on PPµTP by color-saturation convincingly correlates with the absorbance–based assay, it could be an outstanding low-cost substitute to the spectrophotometric method. The minimum amount required for the detection on

PPµTP is 10 ng of protein in 1µl of a solution with an accuracy of 97%. However, such a low amount of protein could not be quantified by the conventional microtiter-based assay technique. Besides, the spectrophotometer needs different filters for different color solutions, whereas the present method is compatible with any color. Also, protein estimation involving BCA and Bradford method is not suitable on paper discs as it reacts with the reagents itself.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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