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Sensitive and Rapid Bradford and BCA Protein Assay by a Common Desktop Scanner

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Abstract

Background: The conventional method of protein quantification involves the need for costly 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.

Aim: In this communication, we report a sensitive image-based protein assay method overcoming the challenges faced by the conventional method.

Method: BCA and Bradford protein assay in a miniaturized form was 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 of expensive equipment for quantification.In this procedure, after the assay, the plate with a color solution was turned upside down for capturing images in a desktop scanner. The image were 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 correlated with the absorbance-based method carried out in a microtiter plate with Pearson correlation coefficients of 0.995178 and 0.981006 for BCA and Bradford assay respectively. A test protein quantified by this method showed 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 which reduced the sensitivity further to the 10 femtogram/test zone. In short, image-based protein assay on PPµTP and UAP platform could be an outstanding alternative either to spectrophotometric or paper-based protein assay.

1. 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 possibly a useful solution for such an assay technique. Prerequisites for an image-based assay 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 disc ELISA assay [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].

In this present work, image-based protein quantification by BCA and Bradford methods on polypropylene micro test plate (PPµTP) using color saturation has been carried out (Figure 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.

2. Material and Methods

2.1. 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 (pH7.2). 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.

2.2. Procedure for making PP µTP 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 then cut into a strip of dimension 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).

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 diameter of the disc was made similar to the diameter of the PPµTP cavity.

2.3. 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 of 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 the ratio of 50:1 as per the supplier's protocol. From this, 100 µl was added to each well-having a 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.

2.4. Spectrophotometric BSA quantitation by 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 microlitres. Bradford reagent (5X) was diluted to 1X using distilled water. Then 200 µl of Bradford reagent (1X dilution) was loaded to 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 iMarkTM Microplate Reader, USA).

2.5. Image-based BSA quantitation by BCA and Bradford methods

The image-based analysis was done using the same colored assay solution as obtained from section 2.3. and 2.4. 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 [17]. 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 concerning BSA concentrations. We have chosen saturation as a color space because it gives a good correlation with absorbance as reported in our previous paper [18].

2.6. 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 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 section 2.5. Now, different amounts of chicken egg albumin (25 and 40 µg/were was 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 amounts of chicken egg albumin were estimated.

2.7. 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 along 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 chicken egg albumin (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 in section 2.5.

2.8. Protein quantification on Ultraminiaturized assay plate (UAP)

BSA standards by BCA procedure were made on UAP (1µl volume plate) 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 (chicken egg albumin) 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 along 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.

3. Results and Discussion

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. Figure 2 shows a strong correlation among color-saturation and absorbance concerning BSA concentrations with a Pearson correlation coefficient of 0.995178 and 0.981006 for BCA and Bradford assay respectively.

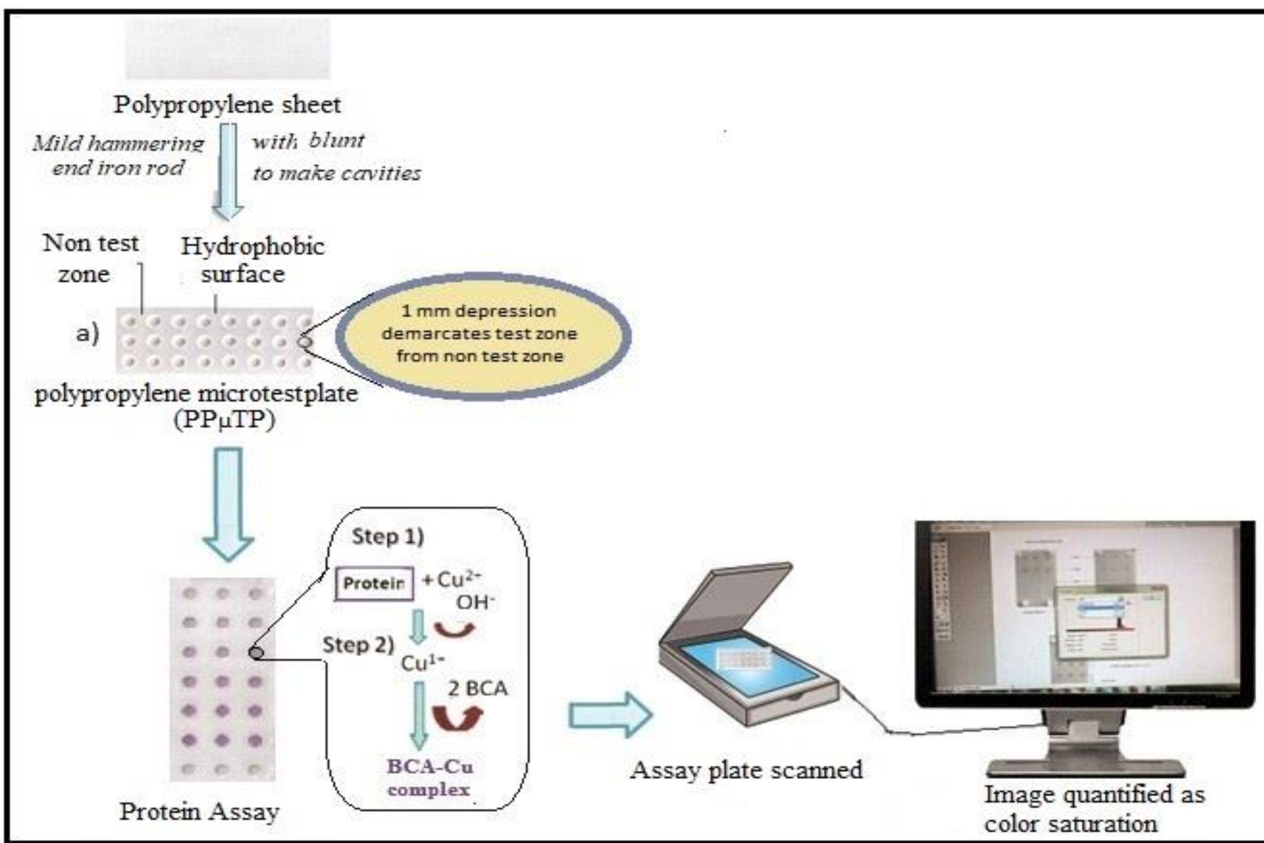

Figure 1. Overview of an image-based protein assay

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 the paper itself reacts with both the protein assay reagents, giving erroneous results. Also, the color disappeared 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, have made BSA standards for both methods. From this standard (Figure 3), we have quantified different concentrations (25 and 40 μg/ ml) of chicken egg albumin (CEA) on PPμTP. BCA method on PPμTP shows 26 and 40 μg/ ml of CEA (Figure 3a) whereas the Bradford method shows 24.5 and 38 μg/ ml of CEA (Figure 3b). However, 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 (Figure protein. 3c) which was performed in a microwave oven.

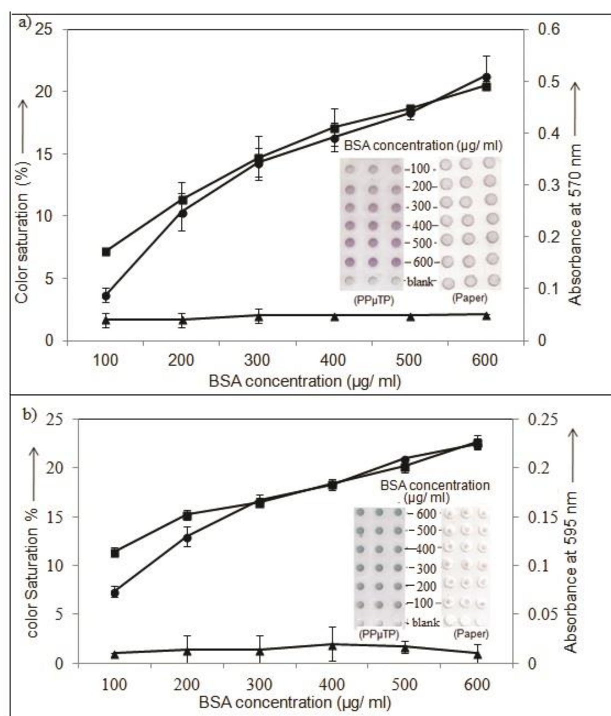

Figure 2. Absorbance and color-saturation-based BSA quantitation by (a) BCA and (b) Bradford procedures. Absorbance values (■) were recorded in a spectrophotometer. Color- saturation-based quantitation was done on PPµTPs (●) and paper discs (▲) from the scanned images (inset) of the same assay solutions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

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 (actual amount of 25 µg/ ml). Image-based protein assay was further carried out on a 1 µl volume plate [19]. Different concentrations of BSA on UAP were assayed to make a standard graph (Figure 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 this standard graph and calculated amount of analyte protein.

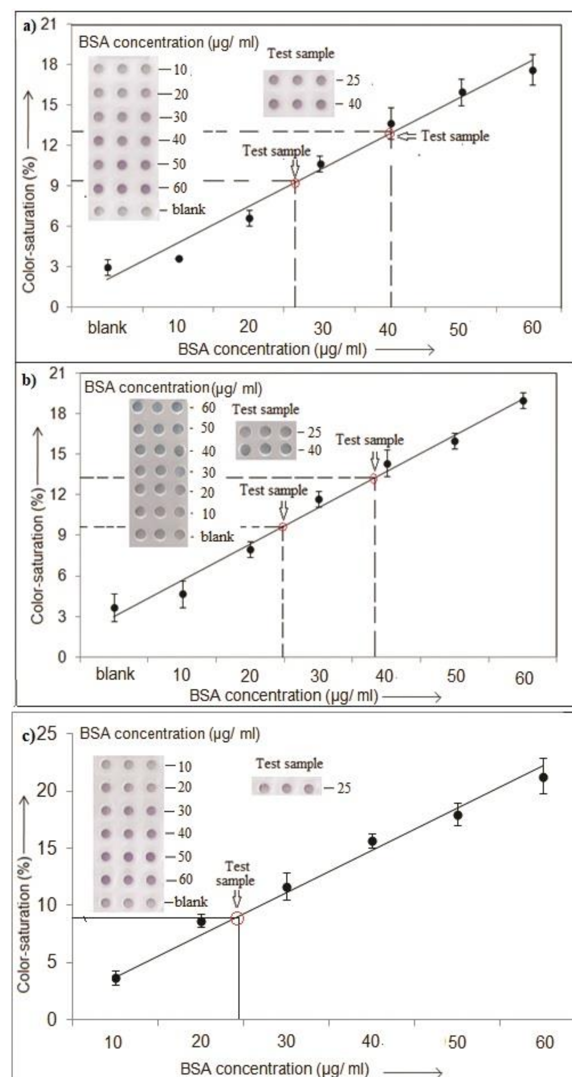

Figure 3. Quantification of a test protein (chicken egg albumin) from BSA standards made by (a) BCA (b) Bradford procedures on PPµTP via color-saturation and c) by microwave-mediated protein estimation on PPµTP by BCA method.

Figure 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 detects 10 femtograms protein per test zone; therefore could be an important tool to quantify proteins that are precious or not available in plenty.

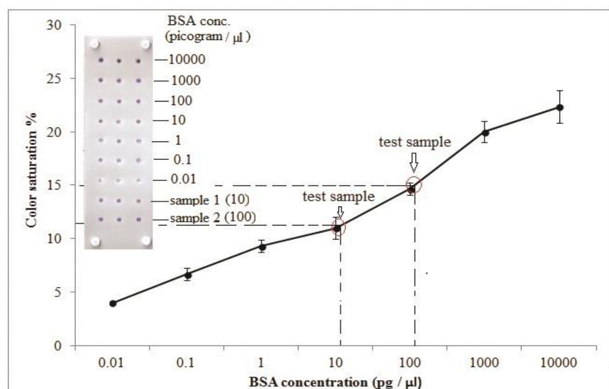

Figure 4. BSA standard graph is made using different concentrations of BSA and quantifying them by color saturation values on a miniature assay plate. Quantification of the test protein is determined by putting the color saturation values on the BSA standard graph after its assay.

4. Conclusions

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 detection of 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 disc as it reacts with the reagents itself.

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6. References:

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