Measurement of Protein in Latex Glove Using Computerized Colorimetric Protein Estimation Method

C. K. Toa, *Member, IAENG*, K. S. Sim, *Member, IAENG*, K. L. Mok and Y. K. Chan

***Abstract*—Proteins of the latex glove will become a potential allergy threat to people who are frequently exposed to latex. A robust way to control protein content is by estimating the amount of protein. Several established protein estimation methods such as modified Lowry method and smartphone for point-of-care quantification of protein method have been proposed. However, most of the conventional methods involve many chemical processes, and time-consuming. In order to overcome those limitations, a novel method named computerized colorimetric protein estimation (CCPE) is designed. The glove sample goes through chemical test to perform protein detection. Later, the sample is converted into digital image through scanning process by using flatbed scanner. A new algorithm named smoothing colorimetric adaption (SCA) has been proposed to perform image filtering on smoothing the wrinkled surface of glove and color difference formula to calculate the delta E, ΔE data of glove sample. The experimented data is then plotted in a standard curve and validated with the protein concentration data from the Malaysia Rubber Board (MRB). Based on the results, a strong correlation with R^2 percentage equal to 97% is established between both data. This indicates that the CCPE method provides reasonably practicable estimation of the protein concentration.**

***Index Terms*— Computerized colorimetric, Color difference, Latex glove, Protein concentration**

I. Introduction

Latex medical gloves are made from natural rubber latex (NRL) [1], which is a raw material that comes from Hevea Brasiliensis rubber trees [2]. In the healthcare sector, latex gloves are medical accessories used to protect patients and healthcare workers from exposure to bloodborne pathogens and other harmful substances through contact with bodily fluids [3], [4]. Besides, those gloves also act as a protective screen against the transmission of virus and bacteria to the human skin. It provides excellent barrier protection with great touch sensitivity and flexibility. Because of the excellent elastic properties, medical gloves have been used favorable worldwide for years.

Even though latex gloves have been seen as a significant piece of equipment in the healthcare sector, a prevailing issue in question is the possibility of causing latex allergic reaction [5], [6]. The latex allergy is called Type I latex hypersensitivity which is an IgE (immune) mediated reaction due to the proteins found in the NRL. Reactions to the allergy will usually show after exposure to the latex for a longer period of time. The most acute and life-threatening reaction is an anaphylactic shock which can cause death if left untreated. In the medical field, it is a habit for healthcare workers to frequently wear latex gloves, given that it can act as a barrier protection against the dangerous microorganism [7]. Thus, it becomes an unavoidable case for those workers to have a high risk of developing latex allergy. Based on research, it was found that latex gloves with higher protein concentration will easily cause an allergic reaction. So, by reducing the amount of proteins inside the latex glove, the individual will have the lowest possibility of developing latex allergy. This can be done by continuously leaching the glove to eliminate the proteins as much as possible during the process [8]. The Food and Drug Administration (FDA) and American Society for Testing and Materials (ASTM) allow claims of 50 μg/g as the lowest protein concentration for latex medical gloves [9].

In order to know the amount of protein inside the glove, a protein estimation method is a must. Several conventional methods such as a smartphone for point-of-care quantification of protein [10], modified Lowry method [11], maximum minimal variation (MMV) method [12], and immunochemical method [13] have been developed. Those methods use the colorimetric technique where it determines the protein concentration based on the color compound of the solution. Generally, those methods are complex in the chemical process, time-consuming, require sophisticated technology, and need to be operated by skilled technicians. Due to the limitations, those methods were not efficient for continuous data collection and development.

In this paper, a new method named computerized colorimetric protein estimation (CCPE) has been proposed to overcome the limitation of conventional methods. A detailed process of protein detection chemical test, image filtering, and color difference calculation were performed to obtain analytical data in estimating the protein concentration of gloves. The objective of this paper is not to replace the modified Lowry methods, but proposing an analytical protein estimation platform with complementary advantages to the process of modified Lowry method.

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II. Related Work

A standard method to evaluate the latex allergy is through the estimation of protein concentration. Few conventional protein estimation methods have been employed to estimate the protein concentration of latex gloves.

A. Smartphone for point-of-care quantification of protein

Point-of-Care (POC) method [10] serves as an alternative method for a spectrophotometer in the detection of protein solution. The POC method presents a low cost (approximately US$ 700.00) and simplicity as compared to a spectrophotometer. This method uses smartphone-based optical detection to perform the analysis. There are two components required for performing optical detection which are negatoscope (source) and smartphone (radiation detector). The negatoscope is emitting the white light to the bottom of 96-well plates. After that, the light will pass through the sample solution and received by a smartphone digital camera in the form of the image. The hue, saturation, value (HSV) color model is applied to achieve a linear fitting between the sample image and the concentration of Bovine Serum Albumin (BSA). The process and standard graph of POC method are performed as shown in Figure 1 and Figure 2. During the process, the image of the solution is captured in the darkroom where there is no interference of external radiation. The result shows that the protein data is obtained with an accuracy of 93%.

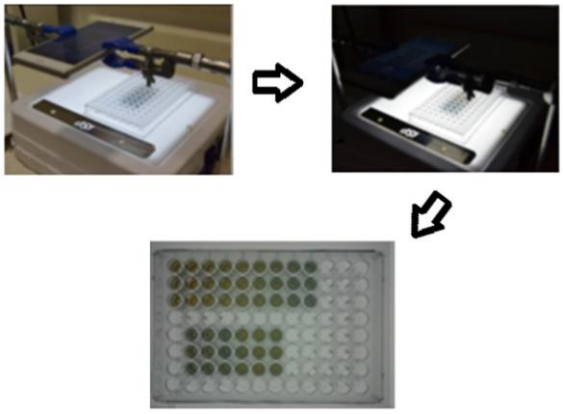


Fig. 1. Process of POC method in determination of protein

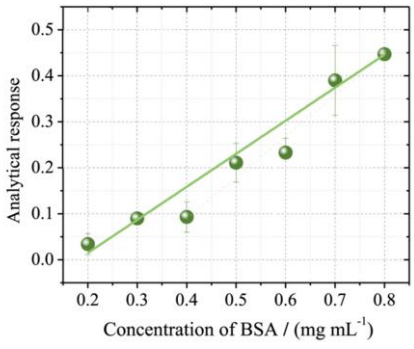


Fig. 2. Graph for the estimation of protein using POC method

Even though the POC method has complementary advantages to the spectrophotometer in terms of cost, but the chemical process for this method is still complicated, tedious and costly. It needs to use more than one chemical reagent to perform the chemical test on detecting the protein in the solution. The process of the chemical test also needs to be done manually by a skilled worker since it involves chemical reaction in the solution.

B. Modified Lowry method

The American Society for Testing and Materials (ASTM D5712) standard test method [14] for analysis of aqueous protein concentration in latex glove was using the modified Lowry method [10], [15]. This method uses a biochemical assay called folin–ciocalteu reagent for determining the protein concentration in a solution. The soluble protein can be analyzed through the color changes in the solution. The color of the solution is then measured and calculated using the spectrophotometer. According to the formulation of improvement modified Lowry method, single absorbance (without $CuSO_4$), T_{single}, mix absorbance (with $CuSO_4$), T_{mix} and compound, $T_{compound}$ are used to calculate the value of protein absorbance ($T_{protein}$) by the Equation 1:

$$T_{\text{total protein}} = 1.25\left(T_{\text{mix}} - T_{\text{single}}\right) \quad (1)$$

Figure 3 shows the procedure of the modified Lowry method. The latex glove sample is mixed with the Phosphate-Buffered Saline (PBS) solution in order to extract the protein from the latex glove. Later, the protein solution goes through a series of chemical reagents for colorimetric analysis. Finally, the measurement of protein solution is carrying out at 750 nm using the spectrophotometer to calculate the absorbance data. The experiment data is then plotted in the standard graph to estimate the protein concentration as shown in Figure 4. The result shows that the modified Lowry method has an accuracy of 99% on estimating the protein concentration. For your information. the spectrophotometer is an instrument used to analyze the light intensity of the solution over a specific color spectrum wavelength.

The main limitation of the modified Lowry method is the time taken to do a full analysis of protein concentration. Since the chemical process is complicated and tedious, it needs more than 6 hours to complete the process of measuring the protein concentration. The process must be done manually by a skilled technician. Another limitation of the modified Lowry method is the cost of the chemical and instrument. Since the analysis needs to use a lot of different chemical reagents to accurately measure the protein, there will be a relatively high cost of chemicals used throughout the process. Another concern will be the relatively high cost of the spectrophotometer (US$ 10,000.00) used to measure the color changes of the solution.

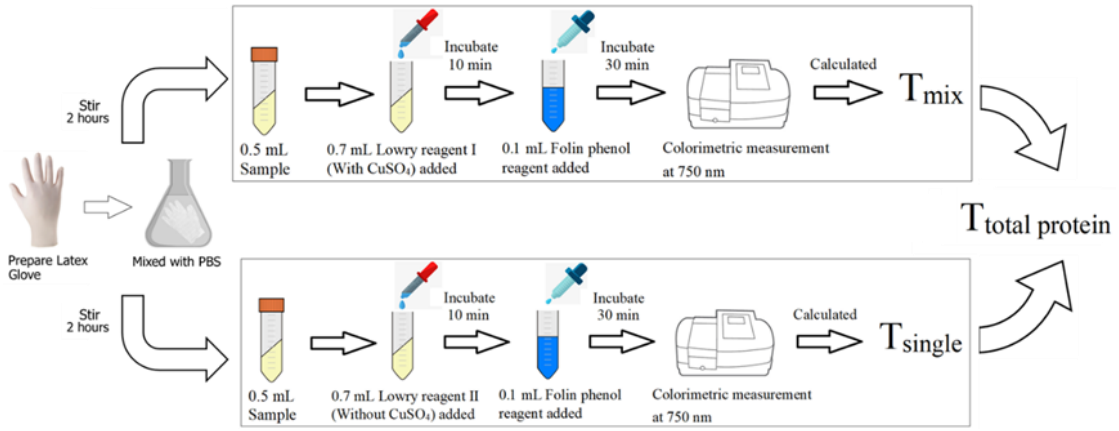


Fig. 3. Procedure of the improvement modified Lowry method

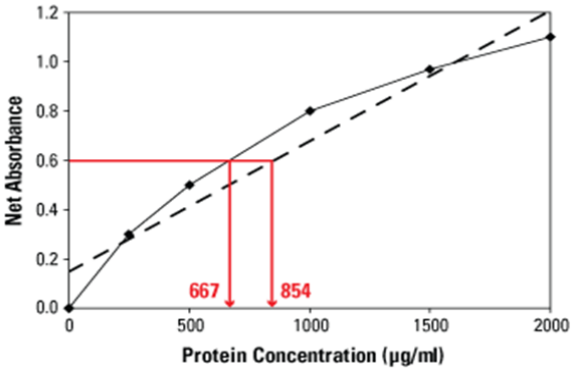


Fig. 4. Standard graph for the estimation of protein concentration using modified Lowry method

III. Proposed Method

The computerized colorimetric protein estimation (CCPE) method is proposed to estimate the protein concentration of the glove. The process of the proposed method is divided into two phases. The first phase conducts protein detection test and the second phase performs colorimetric analysis on the glove sample.

A. Protein detection chemical test

The procedure of the protein detection chemical test is shown in Figure 5. The process of conducting the chemical test is unlike any other conventional method. For those conventional methods, the detection of protein is through the reaction of the chemical reagent with the protein solution of the glove. The solution can be extracted by mixing the glove with a buffer such as Phosphate-buffered saline (PBS). For the proposed method, instead of extracting solution from the glove, the chemical reagent reacts directly with the glove sample.

Our method uses Bradford protein assay reagent as a chemical reagent to detect the proteins located on the gloves [14]. The beige color of the glove sample will turn into blue color with the presence of protein on the glove surface. After the chemical test, the raw sample and the chemical bound sample are placed on top of the analytical balances manual to measure the weight. After that, the sample is digitally converted into a digital image through a scanning process by using a flatbed scanner.

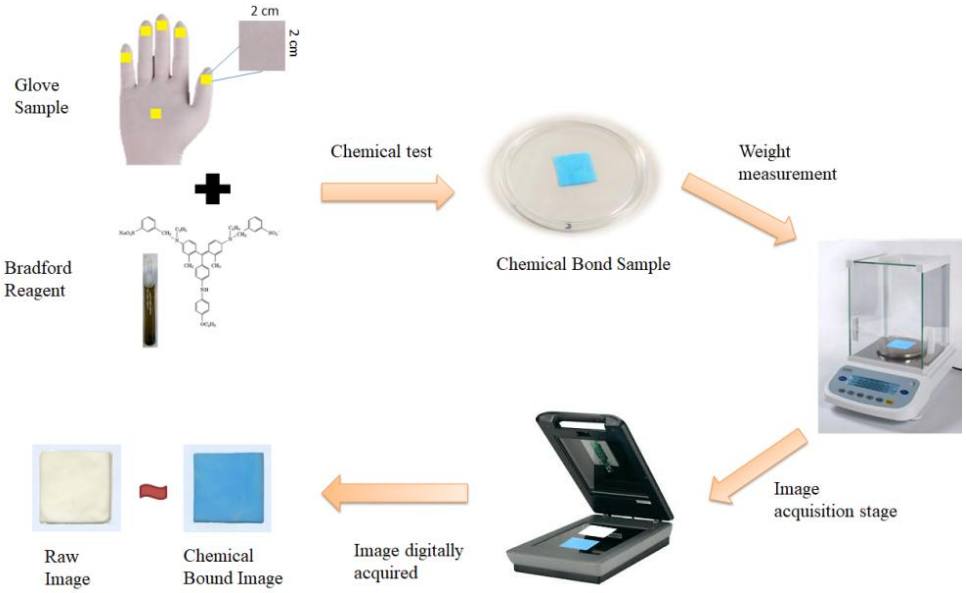


Fig. 5. Process for protein detection chemical test

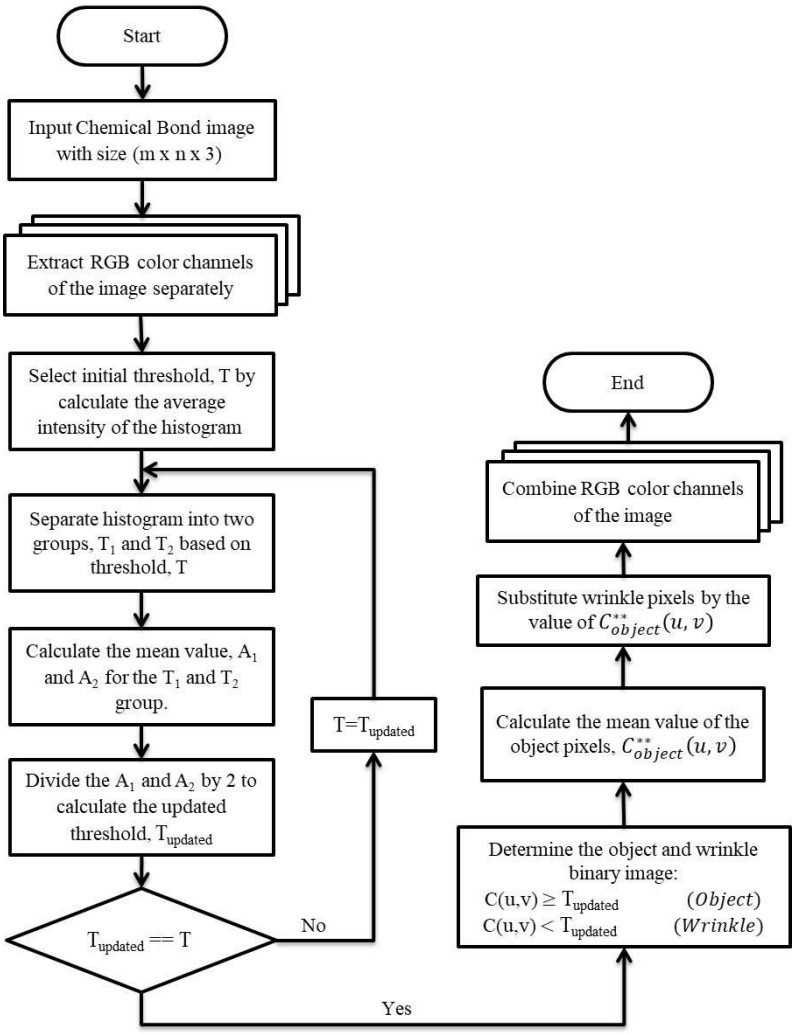


Fig. 6. The flow chart of wrinkle filtering

B. Colorimetric analysis using smoothing colorimetric adaption (SCA) algorithm

After obtaining the digital image of the raw sample and chemical bound sample, the next phase is to apply a colorimetric analysis on the images by using the smoothing colorimetric adaption (SCA) algorithm. Generally, the proposed algorithm consists of two processes. First is performing image filtering on the wrinkle of the sample using image thresholding and morphology constrain [16]-[18]. After the preprocessing on the image, the second process is to calculate the delta E value (ΔE) for the color difference between raw sample and chemical bound sample.

1. Image filtering on wrinkle sample

The wrinkled surface is a vital characteristic as it can negatively influence the calculation of the color difference in the sample image. Thus, the image processing needs to be performed in order to filter out the wrinkle on the surface of the sample before proceeding to the next process. Figure 6 shows the flow chart of the wrinkle filtering.

Based on Figure 6, the chemical bound image, C(u,v) is separated into red (R), green (G), and blue (Blue) components with the same size. Each component goes through thresholding in order to extract the object from the background in the image. To automatically calculate the threshold value, the following steps have been taken:

(1) Convert the R, G and B components into a histogram and calculate the average intensity as shown in Equation 2.

$$T = \frac{I}{2} \tag{2}$$

where I is the intensity value of the histogram, and T is the initial threshold.

(2) Separate the histogram into two groups, T_1 and T_2 based on the threshold, T. The T_1 represents the object image and T_2 represents the background image.

(3) Calculate the mean value of the object (A_1) and background (A_2) for T_1 and T_2 groups using Equation 3 and 4.

$$A_1 = \frac{\sum_{(u,v)} C_{object}(u,v)}{Numberof\ Objectpixels} \tag{3}$$

$$A_2 = \frac{\sum_{(u,v)} C_{background}(u,v)}{\text{Number of Background pixels}} \tag{4}$$

(4) Calculate the updated threshold ($T_{updated}$) by finding the

average value of A1 and A2.

$$T_{updated} = \frac{\mathrm{A1} + \mathrm{A2}}{2} \tag{5}$$

(5) Compare the threshold value of T and $T_{updated}$. Repeat step 2 to step 4 until T ==$T_{updated}$.

The threshold value is then used to determine the object and background of R, G and B images.

$$C^*(u,v) = \begin{cases} 0 & \text{if } C(u,v) \geq T_{\text{updated}} \\ 1 & \text{if } C(u,v) < T_{\text{updated}} \end{cases} \tag{6}$$

where C(u,v) and C* (u,v) are the greyscale images. The value of one (1) indicates the wrinkle region of the sample ($C_{wrinkle}$*(u,v)), while the value of zero (0) indicates the object region of the sample (C_{object}* (u,v)). The image will proceed through the morphological process to remove noise on the binary region by considering the structure and form of the image.

Finally, the mean value of object pixels (C_{object}**(u,v)) is calculated using Equation 7. The value of $C_{wrinkle}$*(u,v) is then substituted by C_{object}**(u,v) to filter out the wrinkle region.

$$C^{**}_{object}(u,v) = \frac{\sum_{(u,v)} C^*_{object}(u,v)}{\text{Object pixels}} \tag{7}$$

2. Color difference calculation

After filtering out the wrinkled surface of the sample, the next process is to calculate the color difference between raw sample and chemical bound sample. The sample image is first converted into RGB histogram and each bin represents a different grey level, which carries difference intensity information. That information is important as it is used to calculate the color dominant of the digital image. To determine the repetitive for difference in grey level, a two-dimensional histogram is constructed by consolidating the RGB component into a single histogram as shown in Figure 7.

During the process of consolidating, the intensity value of RGB components will be converted into an index value. Each bin of the index level in the histogram represents the number of repetitive for the RGB value. The highest repetitive value represents the most frequently occurring color which indicates the dominant color in the sample image. The dominant color represents the location of protein that resided in the latex sample.

After performing consolidate procedure, RGB image must be converted into CIELAB to calculate the color difference of image. The CIELAB consists of three numerical values, L* for the lightness, a* for green-red color components and b* for blue-yellow color components. The CIELAB is designed to be uniform with the human color visual system where the amount of change in L*, a* and b* value is the same as the amount of change in human visual perceptive.

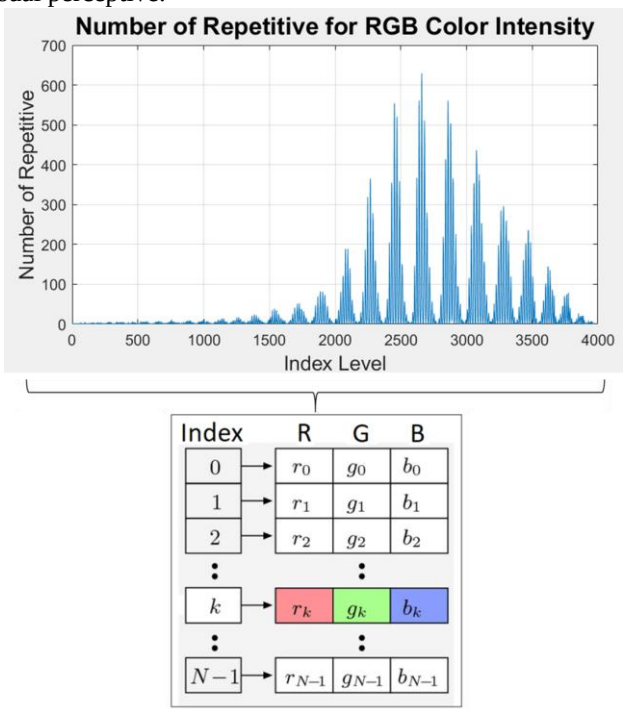


Fig. 7. Repetitive value of RGB color value

Before converting to CIELAB, the RGB color values must first be transformed into XYZ tristimulus values. Equation 9 shows the linear transformation of RGB value before proceeds to XYZ formulation.

$$T \in \{R, G, B\} \tag{8}$$

$$T' = \begin{cases} \frac{T}{12.92} & \text{for } T \leq 0.04045 \\ \left(\frac{(T+0.055)}{1.055}\right)^{2.4} & \text{for } T > 0.04045 \end{cases} \tag{9}$$

where T is a set of RGB color values in the range of [0.0…1.0], and T' is the linearized representation of T with linear RGB value. After that, the color values of R', G', and, B' are substituted into Equation 10.

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} 0.41245564 & 0.3575761 & 0.1804375 \\ 0.212672 & 0.7151522 & 0.0721750 \\ 0.01933399 & 0.1191920 & 0.9503041 \end{bmatrix} \begin{bmatrix} R' \\ G' \\ B' \end{bmatrix} \tag{10}$$

where X, Y, and Z are tristimulus values and the 3 x 3 matrix is the transformation matrix from R' G' B' to XYZ with the D65 reference white. Later, the tristimulus values are converted to CIELAB by the following equations:

$$L^* = \begin{cases} 116\left(7.787\frac{Y}{Y_k} + 0.138\right) + 16 & \text{for } \frac{Y}{Y_k} \leq 0.008856 \\ 116\sqrt[3]{\frac{Y}{Y_k}} - 16 & \text{for } \frac{Y}{Y_k} > 0.008856 \end{cases} \tag{11}$$

$$a^* = \begin{cases} 500\left[\left(7.787\frac{X}{X_k} + 0.138\right) - \left(7.787\frac{Y}{Y_k} + 0.138\right)\right] & \text{for } \frac{X}{X_k} \leq 0.008856 \\ 500\left[\sqrt{\frac{X}{X_k}} - \sqrt[3]{\frac{Y}{Y_k}}\right] & \text{for } \frac{X}{X_k} > 0.008856 \end{cases} \tag{12}$$

$$b^* = \begin{cases} 200\left[\left(7.787\frac{Y}{Y_k} + 0.138\right) - \left(7.787\frac{Z}{Z_k} + 0.138\right)\right] & \text{for } \frac{Z}{Z_k} \leq 0.008856 \\ 200\left[\sqrt{\frac{Y}{Y_k}} - \sqrt{\frac{Z}{Z_k}}\right] & \text{for } \frac{Z}{Z_k} > 0.008856 \end{cases} \tag{13}$$

where X_k, Y_k, and Z_k are the normalized tristimulus value of

a specified white object. After converting the RGB image into to L*, a*, and b* value, the next step is calculating the delta E, ΔE which is the color difference between raw and chemical bound images. ΔE of CIEDE2000 is used to calculate the magnitude of color difference distance between two colors with the same size. Two different colors of glove sample denoted by 1 (raw image) and 2 (chemical bond image) are calculated using Equation 14-17:

$$\Delta L^* = L_2^* - L_1^* \tag{14}$$

$$\Delta C^* = \left(a_2^{*2} + b_2^{*2}\right)^{0.5} - \left(a_1^{*2} + b_1^{*2}\right)^{0.5} \tag{15}$$

$$\Delta h^* = 2\sqrt{\left(a_1^{*2} + b_1^{*2}\right)^{0.5} \cdot \left(a_2^{*2} + b_2^{*2}\right)^{0.5} \sin(x)} \tag{16}$$

$$x = \frac{1}{2}\left(\tan^{-1}\left(\frac{b_2^*}{a_2^*}\right) - \tan^{-1}\left(\frac{b_1^*}{a_1^*}\right)\right) \tag{17}$$

where is ΔL*, ΔC* and Δh* are the differences in lightness, chroma, and hue between raw and chemical bound images. After that, L*, a*, and b* values of the images also used to calculate the weighting functions by the following equations:

$$\overline{L}^* = \frac{(L_1^* + L_2^*)}{2} \tag{18}$$

$$\overline{C}^* = \frac{(C_1^* + C_2^*)}{2} \tag{19}$$

$$\overline{h}^* = \frac{(h_1^* + h_2^*)}{2} \tag{20}$$

$$S_L = 1 + \frac{0.015\left(\overline{L}^* - 50\right)^2}{\sqrt{20 + \left(\overline{L}^* - 50\right)^2}} \tag{21}$$

$$S_C = 1 + 0.045\overline{C}^* \tag{22}$$

$$S_H = 1 + 0.015\overline{C}^*\left(1 - z_1 + z_2 + z_3 - z_4\right) \tag{23}$$

$$z_1 = 0.17\cos\left(\overline{h}^* - 30\right) \tag{24}$$

$$z_2 = 0.24\cos\left(2\overline{h}^*\right) \tag{25}$$

$$z_3 = 0.32\cos\left(3\overline{h}^* + 6\right) \tag{26}$$

$$z_4 = 0.20\cos\left(4\overline{h}^* - 63\right) \tag{27}$$

where S_L, S_C, S_H are the weighting functions account for variation in visual color difference sensitivity. Later, those values are substituted into Equation 28 and 29 to calculate the color difference delta E, ΔE.

$$R_T = -2\sin\left(-60\exp\left[-\left(\frac{\overline{h}^* - 275}{25}\right)^2\right]\right)\left(\frac{\overline{C}^{*7}}{\overline{C}^{*7} + 25^7}\right)^{0.5} \tag{28}$$

$$\Delta E = \sqrt{\left(\frac{\Delta L^*}{S_L}\right)^2 + \left(\frac{\Delta C^*}{S_C}\right)^2 + \left(\frac{\Delta h^*}{S_H}\right)^2 + R_T\left(\frac{\Delta C^*}{S_C}\right)\left(\frac{\Delta h^*}{S_H}\right)} \tag{29}$$

where R_T is a rotational function that is used for weighted chroma difference and hue difference in the blue region.

IV. Result and Discussion

A. Comparison of conventional method and the proposed method

In this study, more than a hundred pieces of latex gloves were used for the experiment. They varied from low to high protein concentrations. Table I shows the performance of the proposed method as compared with the conventional methods.

TABLE I
PERFORMANCE OF THE CONVENTIONAL METHODS AND PROPOSED METHOD

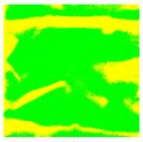
Features \ Methods	**Modified Lowry**	**Point-of-Care (POC)**	**Computerized Colorimetric Protein Estimation (CCPE)**
Types chemical reagent usage	Using numerous types of reagent	Using numerous types of reagent	One type of reagent
Protein detection approach	Through the reaction of the chemical reagent with the aqueous protein solution	Through the reaction of the chemical reagent with the aqueous protein solution	Chemical reagent directly bind with the glove sample itself
Appearance of latex protein	The colour of solution will turn into blue	The colour of solution will turn into blue	The colour of glove sample surface will turn into blue
Scientific Measurement	Spectrophotometric	Colorimetric	Colorimetric
Equipment	Centrifuge, Machine Shaker, Vortex Machine, Analytical Balances, Spectrophotometer, Computer	Centrifuge, Machine Shaker, Vortex Machine, Analytical Balances, Negatoscope, Computer	Analytical Balances, Scanner, Computer
Processing time to complete test for one glove model	More than six hours	More than six hours	Need only one hour
Technical skill in chemistry and chemistry-based technology	Professional skills	Professional skills	Basic skill
Analytical data	Absorbance (Quantity of light)	Hue, Saturation, and Value (HSV)	CIEDE2000 colour difference value

Based on the Table I, we observed that the conventional methods such as modified Lowry and point-of-care (POC) were applying numerous types of chemical reagent such as phosphate-buffered saline (PBS), bovine serum albumin (BSA), phosphotungstic acid, sodium hydroxide, and folin–ciocalteu reagent as compared to computerized colorimetric protein estimation (CCPE) method which applied only Bradford reagent. For the protein detection approach, modified Lowry and POC method went through the reaction of the chemical reagent with the aqueous protein solution, while the CCPDE method is using the chemical reagent to directly bind with the glove sample itself. Besides, those conventional methods also using numerous amount of equipment to perform the protein estimation test. Due to great amount of chemical reagent and equipment usage, it makes the process become complicated, costly and time-consuming. It also needs a technician with professional skills to successfully operate the whole process. For CCPE method, since it only using one chemical reagent and few amounts of equipment, we only need a technician with basic skills to smoothly complete the protein estimation test in one hour. For analytical data, each method produces different types of data to estimate the protein concentration of the glove. For modified Lowry method, it calculates the absorbance value by measure the quantity of light absorbed by a sample using spectrophotometer, while the POC method converts the Red, Green, Blue (RGB) value of the sample image into Hue, Saturation, and Value (HSV) value. As for the CCPE method, it calculates the ΔE value using the International Commission on Illumination Delta E (CIEDE2000). Since the analytical data for each method is different, we will validate the accuracy of the CCPE method using coefficient of determination (R^2).

B. Validation of the computerized colorimetric protein estimation (CCPE) method

During the process of CCPE method, the glove samples which appeared to have a wrinkle on the surface will go through image filtering and the result is shown in Table II.

TABLE II
WRINKLE FILTERING ON SAMPLE IMAGE

	Sample Image (Before filtering)	Filtering algorithm	Sample Image (After filtering)
Sample 1	 (a)	 (b)	 (b)
Sample 2	 (c)	 (d)	 (d)
Sample 3	 (e)	 (f)	 (f)

In Table II (a), the image shows a brown stain mark on the lower-right corner. The mark was due to an incomplete washing process after immersing in the chemical reagent. In order to avoid miscalculation on the color difference, the image was filtered out and the result is shown in Table II (b). Based on the filtered image, the mark has completely disappeared. The same was seen in Table II (c) and (e) which have a wrinkle on the surface image and the result after filtering in Table II (d) and (f). Based on Table II, it shows that image filtering has the capability to filter out not only wrinkle but also stained marks on the surface.

After the process of image filtering, the sample images next went through the calculation of color difference. Based on the calculation, the samples could be divided into three categories of protein concentrations: low level, medium level and high level as shown in Table III. Each protein concentration in the row consists of six samples per glove.

TABLE III
WRINKLE FILTERING ON THE SAMPLE IMAGE

		Latex Glove Sample					
		Thumb	Index	Middle	Ring	Pinkie	Palm
Protein Concentration	Low (20-50 ug/g)						
	Medium (51-150 ug/g)						
	High (>151 ug/g)						

Based on Table III, it is clear that the glove sample images have different blue color saturation for the low, medium and high levels of protein concentration. It appears that samples with low-level protein concentration have low saturation and would gradually increase when reaching the high-level protein concentration. This indicates that the protein concentration of glove sample could change proportionally with the color saturation in the sample.

After calculating the color difference, the accuracy and reliability of ΔE data were verified by plotting the graph as shown in Figure 8. Each data represents ΔE value for the whole glove and it was obtained by calculating the average value of ΔE for six samples (fingertips and palm). The graph shows the correlation between the protein concentration and color difference ΔE. The protein concentration data of the glove sample was provided by the Malaysia Rubber Board (MRB) using the American Society for Testing and Materials (ASTM D5712) standard test method. To validate the accuracy of the proposed method, both protein data and ΔE data must be obtained from the same type of glove. Since the protein concentration unit used was per gram of glove (µg/g), the delta E values were also divided by weight (gram) of glove sample (ΔE/g) to normalize the data.

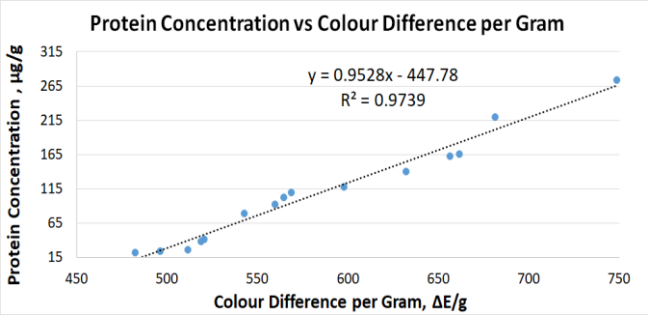


Fig. 8. Correlation between protein concentration and delta E color difference

Based on the graph, we used a linear regression analysis to show that the delta E per gram values changed proportionally with the protein concentration of glove samples. For low protein concentration (20-50 ug/g), the color difference per gram, ΔE/g value is in the range of 480-530, while medium protein concentration (51-150 ug/g) is in the range of 531-640, and for high protein concentration (>151 ug/g) is more than 641.

As such, protein concentration could be easily estimated or extrapolated from corresponding ΔE/g values. The graph shows a good and clear correlation with the coefficient of determination (R^2) of 97% between the protein data and color difference data. By using the equation y=0.9528x-447.78, we can calculate the estimated protein concentration value as shown in Figure 9.

The graph shows that the R^2 value was 0.9734, indicated that the estimated protein concentration value that calculated using the equation was very close to the standard protein concentration value. Hence, the equation can be used to calculate the protein concentration of an unknown glove sample.

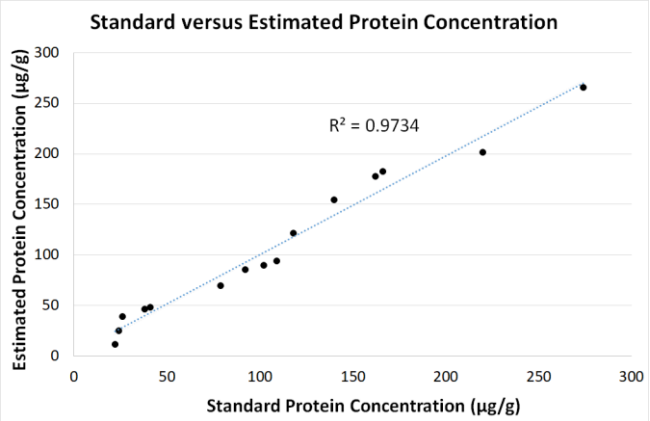


Fig. 9. Graph between standard and estimated protein concentration

Besides that, a paired t-test formulation in Equation 30 has been used to find if there is a significant difference between the means of estimated and standard protein concentration groups. The d in the equation indicates the difference between the two groups and n is the total number for a group. A null hypothesis for the sample t-test has been used, assuming that the means of those two groups were similar to each other.

$$t = \frac{\frac{(\Sigma d)}{n}}{\sqrt{\frac{\sum d^2 - \left(\frac{|\sum d)^2}{n}\right)}{(n-1)(n)}}} \quad (30)$$

At the confidential level of 95%, the calculated t-statistic value using the above equation is 8.67×10^{-4} which is smaller than the critical t-statistic value (2.145) found on two tails T distribution table [19]. This indicated that the null hypothesis was accepted.

We also have used f-test formulation at 95% confidential level as shown in Equation 31 to find out the similarity between the estimated and standard protein concentration.

$$f = \frac{S_1}{S_2} \quad (31)$$

where S_1 is the largest sample variance and S_2 is the smallest sample variance. The null hypothesis of the f-test is having no significant difference between the estimated and standard values. It seems that the f-value which is equal to 1.0324 was smaller than f critical one-tail value (1.6709), therefore the null hypothesis was not rejected.

The significant difference between t-test and f- test is that the former is follows the Student t-distribution, while the latter follows the Snedecor f-distribution. Besides, the application of t-test is to compare the means of two populations, while the f-test is used to compare two population variances. Both t-test and f-test show that there have no significant differences were observed between the data of estimated and standard protein concentration in Figure 9.

Based on those results, it shows that the proposed method has an accuracy of 97% which is higher than POC method, and comparable with modified Lowry method. This

indicates that the CCPDQ method able to estimate the protein concentration of the glove expeditiously and cost-effective as compared to existing methods.

V. Conclusions

The study shows that the proposed computerized colorimetric protein estimation (CCPE) method has the capability to estimate the protein concentration of the latex glove. The result shows that in combination with the Bradford protein assay, the CCPE method is able to measure the amount of proteins in the latex glove successfully. The proposed method is not to replace the conventional methods but proposing an analytical platform that is uncomplicated and straightforward compared to conventional methods. Moreover, the smoothing colorimetric adaption (SCA) algorithm is able to provide satisfactory results on performing filtering on the wrinkle sample image and calculating the color difference value of the images. A high correlation coefficient with R^2 percentage of 97% shows that the calculated ΔE value can be reliably used to estimate the protein concentration level in a glove. Implementing this proposed method can effectively reduce the otherwise tedious process of measuring protein concentration in the latex glove using conventional methods.

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