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Design and fabrication of artificial neural network-digital image-based colorimeter for protein assay in natural rubber latex and medical latex gloves

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ABSTRACT

Digital image-based colorimetry (DIC) using a complementary metal oxide semiconductor (CMOS) camera as a detector coupled with artificial neural network (ANN) was developed for protein assay in natural rubber (NR) latex and medical latex gloves. This method was based on the RGB values (red, green and blue) of different color intensities from the reaction of protein complexes with the modified Lowry reagent. Protein standard solutions and protein in the samples were captured by a single image in the DIC light box. The data was processed by an ANN program to evaluate the RGB value of each digital image. Under the optimum conditions, the amount of protein could be determined in the concentration range of 0–10 μg mL^{-1}. When comparing the results obtained from the DIC-ANN with the spectrophotometric method, there was no statistical difference at 95% confidence level by applying *t*-test at unequal variance. The average mean squared error (MSE) for the protein assay was 0.037. The limit of quantitation by the proposed method (defined as the concentration that could be photographed and processed by an ANN program) was 1 μg mL^{-1}. The proposed method was successfully applied to the determination of extractable protein in NR latex and medical latex gloves and proved to be a convenient, rapid and inexpensive method.

1. Introduction

Natural rubber (NR) latex is a milky fluid obtained from the *Hevea brasiliensis* tree, which is a major agricultural product of Thailand. NR latex composed of *cis*-1,4-polyisoprene is widely used for manufacture of tires, gloves, condom, etc. It is well known that NR latex contains proteins recognized as allergenic proteins, and therefore a small amount of these proteins can give rise to latex allergy [1]. Proteins in NR latex and some rubber products may cause an increase in the incidence of sensitization, adversely allergic reactions, and even death through anaphylactic shock. In addition, proteins have an effect on the stability, quality, and durability of some rubber products, especially in latex gloves because they contact with the skin more than other rubber products. Therefore, the detection of these proteins before rubber production is very important. Currently, there are many techniques that can be effectively used for determining protein, such as the standard test method for the analysis of aqueous extractable protein in NR and its products using the modified Lowry method (ASTM D5712-05) [2]. This method is much more sensitive than the Biuret method and other colorimetric methods [3]. However, this method is based on three steps for protein analysis. The first step is protein extraction from NR and its products followed by protein precipitation and purification. Finally, protein is determined by the spectrophotometry. Hence, standard protein solutions prepared to set up the calibration curve must perform under the same procedures as sample pretreatment of the standard test method which is complicated.

Recently, a new technique called digital image-based colorimetry (DIC) based on measurement of the red, green, and blue (RGB) values of different color intensities will be used instead of spectrophotometry. The value provided to the user ranges from 0 to 255 for each color giving more than 16 million different colors. This technique can be interpreted as a colorimetry by reflecting light [4]. Videlicet, a method where the light, reaching each pixel in an image sensor (charge-coupled device, CCD and complementary metal oxide semiconductor, CMOS), is the light reflected by objects. This passes through three different filters (RGB filters), then they are read by color-analysis software. Thus, this technique is highly suitable for colorimetric reaction. The widespread applications of DIC include the prediction of leaf chlorophyll content [5], water quality measurements [6], clinical measurement of blood glucose [7], identification of natural amino acids [8], and application in analytical chemistry [9–12]. In addition, a novel technique that can improve the efficiency of DIC is artificial neural networks (ANNs) [13]. ANNs are the computerized analog of a biological neural system and they are the important class of pattern recognizer which are useful for a wide variety of applications [14]. The architecture of the ANN model is to develop

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relationships between the input and output data through training on a data set [15,16]. ANNs have attracted the interest of many researches in the field of chemistry as modeling tools for multivariate calibrations [4,13,15–19]. Among neural networks, the most popular is the back-propagation neural network (BPNN) [4,13,17,20–23]. The use of ANNs offers an alternative method to analyze proteins in NR latex.

The main purpose of this research is to study the possibility of CMOS camera for the signal recording and the application of ANNs combined with DIC as a detector for the detection of protein in NR latex and medical latex gloves after color forming by the modified Lowry method without the calibration curve construction.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical grade and all solutions were prepared by using deionized (DI) water (Prima Reverse Osmosis, Maxima water purification system, Elga Ltd., England). The stock protein standard solution at the concentration of 1 mg mL^{-1} was prepared by dissolving 100 mg of albumin from hen egg white (Fluka) in 100 mL phosphate buffer for 2 h at room temperature in a polypropylene container. The solution was filtered through a low protein binding filter paper (0.45 μm) and determined the absorbance at 280 nm using a UV–Vis spectrophotometer (V-650 Series Spectrophotometer, Japan). The absorbance was divided by 0.64 to calculate the actual concentration of the albumin stock solution. The standard protein solution was stored at 4 °C. This solution is stable for 7 days [2].

2.2. Extraction and assay procedure

The standard test method for analysis of extractable protein in NR latex and its products using the Modified Lowry method [2] was used in this research. It was including 3 steps; extraction, precipitation and color developing as describe below.

Extraction procedure: a 0.5 g of NR latex (high ammonia latex, 60% of dry rubber content, Thai Rubber Latex Corporation, Public Company Limited, Thailand) and medical latex gloves (sampling 9 pieces from five brands and cutting into small pieces, at approximate size of 0.5 cm^2) were taken into 15 mL centrifuge tubes. Then, 5 mL of phosphate buffer saline pH 7.4 was added. The specimen was extracted by shaking using vortex mixture (Vortex-genie G-560E Scientific Industries, U.S.A.) at room temperature in the middle level for 120 min. Then the extraction solution was centrifuged (Hettich zentrifugen D-7200 Tuttlingen, Germany) at 2500 rpm for 15 min. Consequently, the extract was filtered through a low protein binding filter paper (0.45 μm) into a 15 mL centrifuge tube to collect the supernatant liquid.

Acid precipitation: a 1 mL of each solution, the extraction buffer (blank), standard protein solution, and the sample extract was placed into 15 mL centrifuge tubes. A 0.1 mL of 0.15% (w/v) sodium deoxycholate (Fluka) solution was added and mixed. The mixture was left for 10 min. Then, 0.2 mL of a freshly prepared solution of 1:1 trichloroacetic acid (Sigma-Aldrich) and phosphotungstic acid (Fluka) was added in order to precipitate the proteins followed by mixing and standing for 30 min. After that, the solution was centrifuged at 5000 rpm for 20 min. The supernatant liquid was decanted and redissolved the precipitates by using 1 mL of 0.1 M NaOH (RCl Labscan Limited).

Color developing and measuring: a 2.5 mL of alkaline copper tartrate solution and 0.3 mL of 50% (v/v) folin-ciocalteu reagent (Sigma-Aldrich) were taken into the protein extract from acid precipitation process. The solutions were waited for 30 min then transferred into the sample cell followed by measuring the absorbance at 750 nm with UV–Vis spectrophotometer and the RGB values with the proposed DIC detector.

2.3. DIC system

A schematic diagram of the system assembled for digital image acquisition is depicted in Fig. 1. A light box was made of a 30 × 20 × 20 cm (width × height × depth) black cardboard to protect the system from outside light. The CMOS camera (Microsoft HD-5000, China) connected with laptop (Lenovo G640, China) was secured on the center floor of the box and in front of the sample cell (quartz cuvette, 10 mm Hellma, U.S.A.) which was located in a sample cell holder made by a white plastic. Two white high intensity LEDs (Thailand) were placed at the camera backside for providing constant light intensity throughout the experiment. The LEDs were powered by a 4.5 V DC from three AA dry cell batteries (Panasonic rechargeable HHR-3LVT, China) and the illumination was adjusted by 10 kΩ variable resistors (Thailand).

2.4. ANN-written program

The ANN-program (under copyright since 2011, Thailand) was developed in-house using Visual Basic 6 Service Pack 6, Microsoft Access 2003, and LeadTools SDK version 12. A program was used to extract the red (R), green (G) and blue (B) values from the images. The RGB values were used as the input to the ANN which was trained with the back-propagation of errors learning algorithm. The structure of the ANN is shown in Fig. 2. This network has three input nodes (one each for red, green and blue), two hidden layers with 11 nodes and one output node (protein concentration). The reason for choosing 11 nodes in the hidden layer is because the output is the concentration of standard protein from 0 to 10 μg mL^{-1}. The pictures of the protein standard solutions and sample solutions stored as "jpeg" (24-bit) compressed files at 1280 × 720 pixels and were cropped by the user to give a homogenous area (raw data with weight of 0–255 for each color)

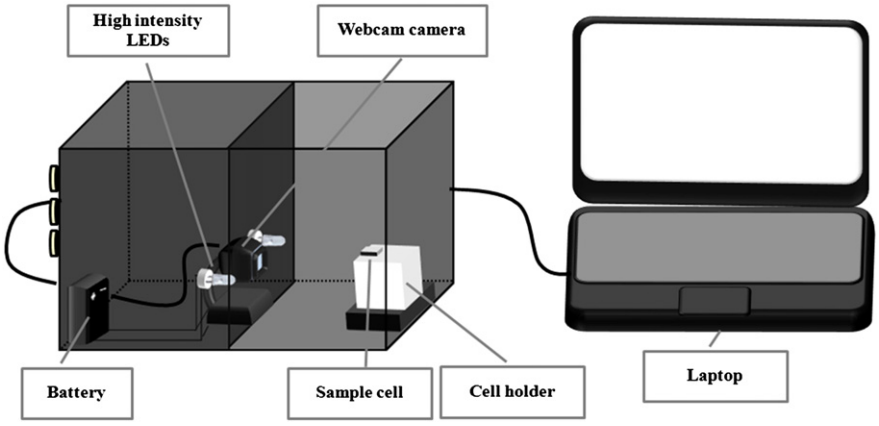

Fig. 1. Schematic diagram of a laboratory-made DIC system.