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Tree-shaped paper strip for semiquantitative colorimetric detection of protein with self-calibration

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ABSTRACT

This paper described a convenient semiquantitative method for colorimetric detection of protein with self-calibration integrated on the test strip. Hydrophilic paper was employed as microfluidic device for running colorimetric assay, tree-shaped design was developed to ensure uniform microfluidic flow for multiple branches. The approach was validated with bovine serum albumin (BSA) colorimetric detection, and colorimetric results observed by naked eyes were consistent with that from apparatus. The device could be coupled with digital transmission of images for remote monitoring system for diagnosis, food control, and environmental analysis.

1. Introduction

Colorimetric detection has been widely applied in analysis of metal [1,2], protein [3,4] and other chemicals [5]. Meanwhile, the modern society highly demands rapid and accurate analysis with convenient operation and low cost [6]. Many materials have been applied as the platform in the field. Liang et al. adopted glass as the base for gold–silver colorimetric detection of protein with high sensitivity [7], Yang and Wang conducted this gold–silver colorimetric detection on a polycarbonate chip [8], Paek et al. established an analytical system for one step immunoassay employing colloidal gold, with a glass-fiber membrane, a cellulose membrane and a nitrocellulose membrane [9]. In particular, Whitesides and co-workers have initiated on paper-based microfluidics devices combined with lithographic technology [6,10] and PDMS plotters [11]. However, no method provided a standard reference in the same assay for direct judgment.

Herein, we have established a new method based on a tree-shaped paper microfluidic device to realize colorimetric detection with simultaneous calibration. Hydrophilic paper was employed as microfluidic device for running colorimetric assays, tree-shaped design was developed to ensure uniform microfluidic flow for multiple branches. To the best of our knowledge, it is the first time to report that integrating the calibration in one assay. Tree-shaped design is easier to fabrication than that with lithographic technology [6], and more convenient to operation than that the sample was dropped in the center of strip towards one step detection [12]. The approach was validated with bovine serum albumin (BSA) in artificial urine colorimetric detection. We test a range from 0 to 5 mg/mL and concentration as low as 0.08 mg/mL could be detected. With this detection range and detection limited, the method could meet the requirements in most of the diagnosis, such as different renal diseases [6]. The colorimetric results were analyzed both by naked eyes and apparatus. With digital camera or cell phone, the resultant images could also be transmitted and analyzed by image software Quantity One.

2. Experimental

2.1. Materials

Cellulose membrane (3 mm, CHR chromatography paper) was purchased from Whatman (Maidstone, England). Bromphenol blue (BPB) was supplied by Nanjing Bookman Biotechnology Ltd. BSA was obtained from Roche Ltd. Citric acid monohydrate was from Nanjing Chemical Reagent Ltd. Other reagents used were of analytical grade.

2.2. Colorimetric assay

Chromatography paper was firstly cut into tree-shaped sheets, with seven branches (3 mm × 45 mm) and a stem (8 mm × 55 mm),

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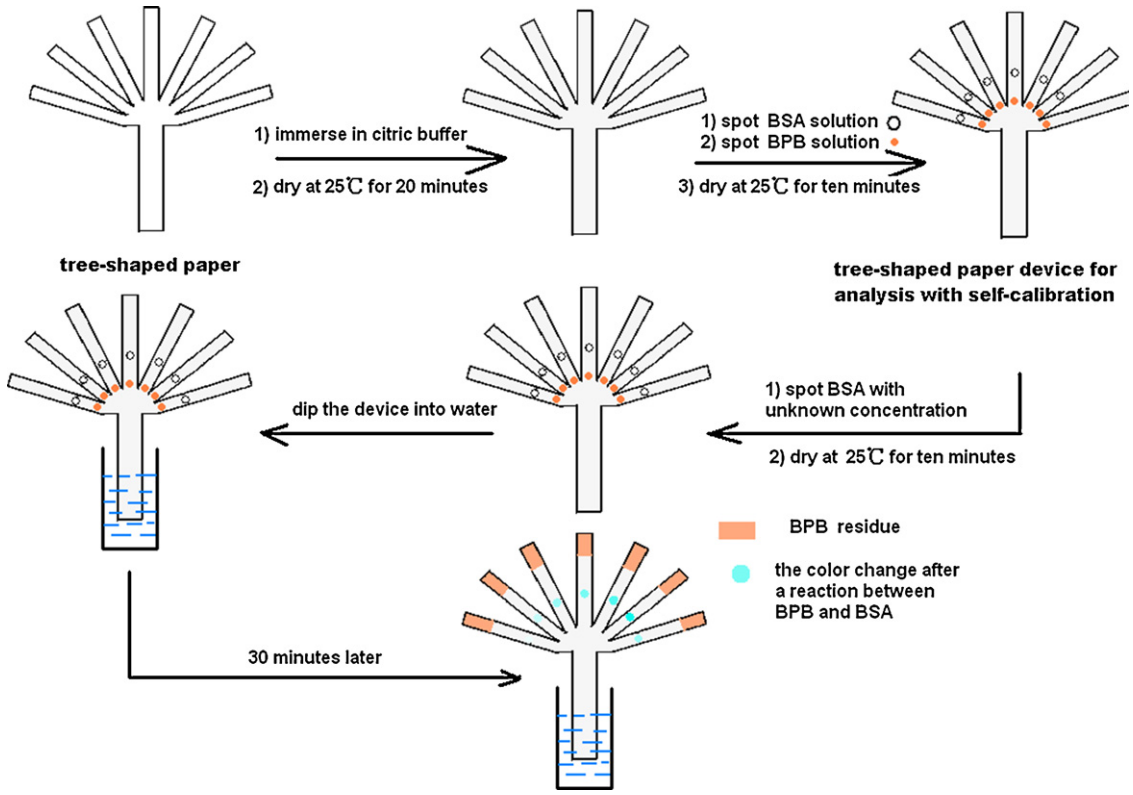

Fig. 1. Diagram of the procedure for tree-shaped device with self-calibration for detection.

which was then immersed into 250 mM citric buffer solution (pH 1.8). The paper sheet was then allowed to dry at 25 °C for 20 min. After that, 0.5 μL of 9 mM BPB solution in ethanol and 0.5 μL of BSA solution were spotted on the sheet respectively (5 mm from the bottom of the branch for BPB and 20 mm from the bottom of the branch for BSA, a series concentration of BSA in PBS buffer were spotted on the left six branches and a BSA in the artificial urine [10] was spotted on the right one). The sheet was then allowed to be dried at 25 °C for 10 min. The procedure of preparation and detection is shown in Fig. 1.

2.3. Colorimetric detection

After the prepared tree-shaped strip were dipped in 6 mL of water for 30 min, different intensities of blue color could be observed. The different concentrations of BSA solution could be judged by eyes semiquantitatively or analyzed the pictures taken by camera or cell phone by Quantity One. In our experimental, camera and parameters are as follows: Olympus FE-210 digital camera with CCD sensor (Tokyo, Japan), maximum resolution 3072 × 2304 pixels, programmed auto exposure without flash light, the object distance is 30–40 cm. These photos were imported into the computer and color differences were analyzed by image software Quantity One (Bio-Rad company). Darkness density of each dot was picked to describe color difference among the volume data in Volume Analysis Report.

3. Results and discussion

3.1. Purpose of self-calibration

The main aim of this research is to integrate a self-calibration on the test strip. With the prepared test strip, the sample information could be collected with standard solution at the same time. The greatest advantage of self-calibration is that affects of environmental conditions (temperature, humidity, pressure, etc) could be minimized to a large extent. In this situation, no consideration of different conditions is necessary to be included and systematic errors can be avoided, and the semiquantitative assay can be achieved immediately. An analytical equipment should provide a standard reference in the same assay for direct judgment when adopted in less developed areas without enough trained medical personnel and sophisticated equipments [13].

3.2. Principle of the tree-shaped design

To validate the idea of self-calibration, a tree-shaped paper device is designed, which could assure the uniformity of micro flows in different branches for multiplex concentration of analytes on the paper. This tree-shaped paper device offers 7 assays, which means a set of 7 data could be collected at the same time, including the color intensity in both the standard solution and the unknown concentration (Fig. 2). When developing reagent water goes from stem to a wider one, its frontline spreads from the previous straight line to a semi-circle. Consequently, in our design all the branches start at the periphery of the same semi-circle, whose center is in the middle of the stem's top; all BSA dots, as well as BPB dots, remain the same distance to the center. This could guarantee equal fluid situation of water in each of the branches and therefore ensures the accuracy of our results. Moreover, this device is made merely by carefully cutting, without any complicated process, such as lithography or building hydrophobic barriers.

3.3. BSA detection

This BSA detection model was adopted from the references [6,10]. The protein assay is based on the nonspecific binding of BPB to proteins. BPB binds to proteins through a combination of electro-

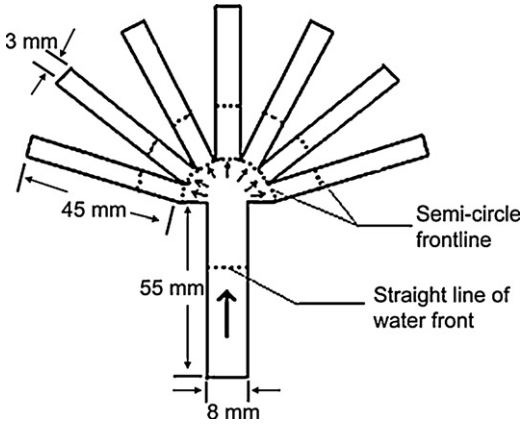

Fig. 2. Tree-shaped design ensures uniform conditions of each assay. The arrow shows the direction of water, indicating that the frontline of the water spreads from a straight line to a semi-circle when coming to a wider channel.

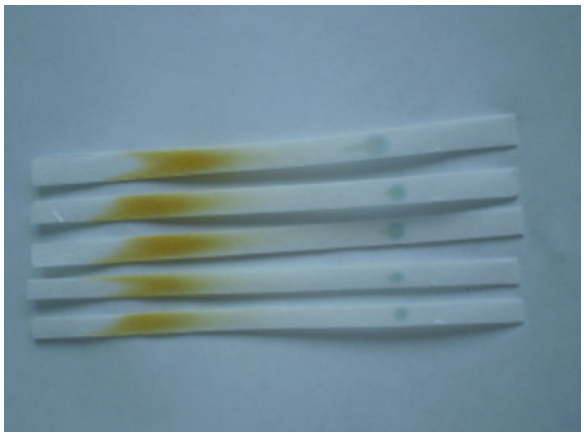

Fig. 3. Assays with BSA samples of the same concentration, but different volumes of 0.3 μL, 0.5 μL, 1.5 μL, 2.0 μL respectively (from the bottom to the top), indicate that volume of the sample solution does not affect the result in large extent.

static and hydrophobic interactions. Under acidic conditions, BPB can interact with BSA to form a stable blue complex. We immobilized samples on the paper strip instead of dipping paper into the sample solution, which could largely reduce the volume of samples needed.

Meanwhile, developing reagent water is an inexpensive, easily accessible and environment-friendly solvent compared to organic solvents commonly used in chromatography. The BPB dotted on the paper runs altogether with water and reacts with BSA, leaving a blue dot where the BSA was immobilized. Color disparities of the blue dots indicate different concentrations of BSA. The remnant of BPB was washed to the end of paper strip by water, rather than staying with the blue dot and thus affecting the analysis of colors.

Moreover, this method does not require an exact volume of samples. Fig. 3 showed that different volumes of samples with the same concentration could present almost the same color density, as long as they could fully spread on paper. This indicates that precise measurement of sample volume could be eliminated when the experiment is conducted in developing countries under rough conditions. According to our observation, the spot would expand with the increase of solution volume, while the color intensity remains unchanged.

In respect to the results, we could either judge with naked eyes or take photos of the paper strips and analyze it with image software Quantity One. The pictures could also be telecommunicated if necessary. All the results are semiquantitative.

3.4. Analysis of BSA in artificial urine

We tested a range from 0 to 5 mg/mL of BSA, and as low as 0.08 mg/mL of sample could be detected by naked eye. The paper

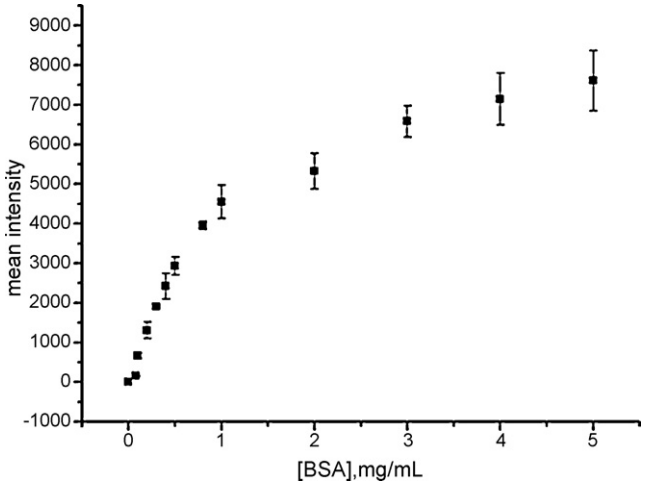

Fig. 4. Standard curve made from the data collected by a camera. Concentrations of BSA were between 0 and 5 mg/mL. Conditions: Olympus FE-210 digital camera, programmed auto exposure without flash light, the object distance is 30–40 cm. Darkness density was picked to analyze by image software Quantity One.

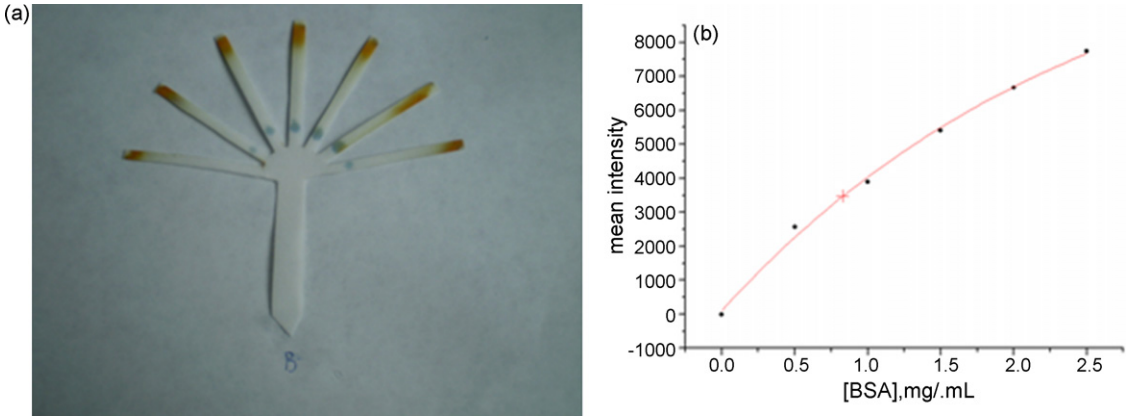

Fig. 5. (a) A tree-shaped paper device with a stem and seven branches after test. (b) A sample detection with a standard curve in the tree-shaped device. The red cross lies in the place of the sample with a mean intensity of 3475, reads 0.82 mg/mL in concentration, while the true value is 0.75 mg/mL. Conditions are the same as in Fig. 4.

strips used for test are prepared in the same way as the tree-shaped device in Section 2.2. A standard curve could be made by image software Quantity One according to the intensities of color in the pictures by a camera (Fig. 4). Similar results could be obtained with a camera cell phone.

Simultaneous calibration was validated by the tree-shaped design (Fig. 5a). The left six assays provides a series of samples with concentration from 0 to 2.5 mg/mL, the right assay is dotted with sample of known concentration BSA with 0.75 mg/mL in the artificial urine. Results can be obtained either from comparison of the color by naked eyes or the intensities in the standard curve made with Quantity One. With naked eyes, we observed in Fig. 4a that the color intensity of the sample in artificial urine (the right assay) was between that of the second and third left assays (that is, between 0.5 and 1.0 mg/mL). With image software of Quantity One, Fig. 5b demonstrates that the color intensity of the sample in artificial urine reads 0.82 mg/mL in the software. The results were consistent with the true value. Only with naked eyes, the accuracy of judgment is determined by the step designed in the calibration. From Fig. 4a, the homogeneous micro fluid situation could also be validated by the phenomena of the same running distance BPB dots.

4. Conclusion

Herein, we established a testing model with self-calibration, achieved by running colorimetric assays on a tree-shaped hydrophilic paper. Results can either be observed by naked eyes or analyzed by software. Cameras and cell phones could help in semi-quantification and telecommunication. Therefore, a simple, inexpensive and rapid method is obtained. Here, we are not only aiming at analyzing the concentration of a certain chemical but also developing a practical approach which could satisfy the growing demands of portable, low-cost and easy operated devices with further application in the areas of medical health and environmental monitoring.

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