Optical Sensing Platform for the Colorimetric Determination of Silver Nanoprisms and Its Application for Hydrogen Peroxide and Glucose Detections Using a Mobile Device Camera

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Silver nanoprisms (AgNPrs) have a unique localized surface plasmon resonance, resulting in strong absorption and scattering within the visible light region. In this work, we propose image acquisition from colloidal solutions of AgNPrs using a combination of transmitted and scattered light. The developed measurement technique could be carried out by separately recording transmitted and scattering images of the solutions, using a mobile device camera prior to a calculation of the empirical absorption value ($I_\lambda$). The $I_\lambda$ value of green for AgNPrs solutions was found to be in agreement with the absorption spectra obtained using a conventional spectroscopic technique. This technique was utilized for the quantifications of hydrogen peroxide and glucose. Good linearity between $\Delta I_{\lambda}$ and those typical analytes were observed. The limit of detection for the typical biosensor of glucose was 19.8 μM. As such, we expect the methodology herein developed for hydrogen peroxide and glucose determinations by means of monitoring the color change of transmitted and scattering images from solutions to contribute to the development of simple, rapid, and reliable detection systems to be further applied to biochemical analysis and clinical diagnosis, as well as to household biosensor applications.

Keywords Silver nanoprisms, localized surface plasmon resonance, colorimetric method, hydrogen peroxide sensor, glucose sensor

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The preparation procedure details are represented in the methodology (with minor modifications for the sake of investigation were prepared following our previously developed protocol). Deionized (DI) water was used in the preparation of all solutions. The AgNPs for colorimetric analysis, and household biosensor applications, reliable method, revealing great potential for biochemical analysis, and the optical characteristic of AgNPs, showed to be a simple, rapid, and quantitative determinations of those chemicals were investigated. We are interested in H$_2$O$_2$ detection because it is broadly used in industrial processes, such as water treatment, electrical circuit cleaning, and medical purposes. Moreover, for some biomolecules, such as glucose and cholesterol, which are the major chemicals to act as the indicators for medical diagnosis, H$_2$O$_2$ is one of products from the enzymatic reaction when they are oxidized by their oxidase enzymes. Since the calculated $I_a$ value was sensitive to the concentration of AgNPs, employing H$_2$O$_2$ etching and correspondent oxidase enzyme reactions, we used this value to quantitatively determine the H$_2$O$_2$ and glucose concentrations. The camera-based detection system developed, based on the optical characteristic of AgNPs, showed to be a simple, rapid, and reliable method, revealing great potential for biochemical analysis, clinical diagnosis, and household biosensor applications.

**Experimental**

**Chemicals**

Silver nitrate, sodium borohydride, starch solution, d-glucose, and glucose oxidase enzymes were all purchased from Sigma Aldrich. Hydrogen peroxide (H$_2$O$_2$) was purchased from Kanto Chemical Co., Inc. Deionized (DI) water was used in the preparation of all solutions. The AgNPs for colorimetric investigation were prepared following our previously developed methodology (with minor modifications for the sake of simplicity). The preparation procedure details are represented in Supporting Information. All glassware and magnetic stirrer bars were rinsed with 2 M nitric acid, cleaned with a detergent, washed with DI water in an ultrasonic bath, and rinsed thoroughly again with DI water prior to use.

**3D-printed analytical device fabrication**

The device was 3D printed (SCOOVO C170, Abee Corporation, Japan) using a black polylactic acid filament. The device was designed and exported using an open-source SketchUp program (Trimble Inc) and for the printer setting, SCOOVO studio was used. The printed device contained a cuvette chamber (10 mm length × 10 mm width × 20 mm height), slots for diffused filters, and chambers for the LED light sources. In order to avoid external light interference, a cap was printed and used for appropriate environment light protection. Inside and lateral views of the printed homemade device, along with a schematic representation of the transmitted and scattering images acquisitions, are shown in Figs. 1A and 1B, respectively.

**Image acquisition and absorption intensity ($I_a$) calculus**

A sample was transferred to a cuvette before being placed into the cuvette chamber. Photographic images were recorded using the camera of a commercial available iPod touch (Apple Inc.). To manually control all parameters, i.e., image acquisition time, ISO, and focal length, Manual Application (Will Global) was used. Images of the cuvettes were acquired from a distance of about 6.0 cm from the camera. For colorimetric analysis of the obtained images, the open-source software ImageJ (National Institutes of Health, Bethesda, MD) was employed. The transmitted intensity ($I_T$) was acquired from a transmitted LED light that had passed through a diffused filter and cuvette before recording the images. The regions of interests (ROIs), composed of a squared region (100 × 100 pixels) of each image, were automatically cropped. Chromatic analyses were carried out on the extracted red, green, and blue channels and were then defined as red ($I_{G}$), green ($I_{B}$), and blue ($I_{A}$) transmitted intensities, respectively. The scattering intensity ($I_s$) was collected from the LED light at a fixed angle of 90°, which had passed through the diffused filter and cuvette (at a bottom) before capturing the scattering image. The extracted red, green, and blue channels were then defined as red ($I_{G}$), green ($I_{B}$), and blue ($I_{A}$) scattering intensities, respectively. The calculated absorption intensity ($I_a$), computed by means of an empirical formula, was then calculated as

$$I_a = -\log\left(\frac{I_s + I_{G}}{I_T}ight).$$

$I_0$ was obtained by measuring DI water through the transmission light path.

**H$_2$O$_2$ sensing by the AgNPs decomposition profile**

Red AgNPs (20 ppm) solutions with the maximum LSPR excitation at 500 nm were mixed with various concentrations of H$_2$O$_2$ at a volume ratio of 1:1 (v/v). The mixed solutions were transferred to a cuvette to monitor the decomposition profile of AgNPs for 1 - 120 min of the incubation time. The relationship between $I_{s}\alpha$ of AgNPs and the H$_2$O$_2$ concentration was investigated.
**Experimental protocol for glucose sensing**

The stock solution of d-glucose was prepared by dissolving 0.01 g of the powder in 10 mL of DI water. d-Glucose solutions with concentrations 20 – 100 μM were prepared by serial dilution of the stock solution with DI water. To investigate the performance of AgNPrs on glucose sensing, 1 mL of 20 ppm AgNPrs was mixed with 50 μL of glucose oxidase enzyme (10.0 mg/L) and incubated for 5 min. A glucose solution of 1 mL was added to the mixture and incubated for 12 h. The absorbance spectrum and an image of the solution were recorded. The relationship between $I_{\alpha}$ of AgNPrs and the glucose concentration was investigated.

**Results and Discussion**

The LED lamp used in this experiment was tested by a comparison with a standard halogen lamp, using the same fiber-optic spectrometer. Figure S1 in Supporting Information shows the LSPR spectra of AgNPrs obtained comparatively from a conventional spectrometer and from our developed chamber using a LED light source. Using a conventional spectrometer, the LSPR spectrum of AgNPrs could detect concentrations of 1 – 20 ppm. The corresponding LSPR spectrum and calibration plot within the same concentration range of AgNPrs could be obtained using the printed homemade device, using LEDs as a light source. This result indicates that the signal from the homemade printed device is reliable, and confirms it can be used for further investigations.

Transmission images of AgNPrs are shown in the top row of Fig. 2A. Transmitted images of AgNPrs at 1 ppm were colorless. A weak red color was observed at 5 ppm of AgNPrs. The red color of AgNPs was stronger when the concentration of AgNPrs was increased. The solid lines in Fig. 2B show the measured $I_t$ values. Red, green, and blue lines represent their own chromatic values. The $I_t$ values of AgNPrs at low concentrations showed high chromaticity levels since the images showed a white background of the diffused filter. When the concentration of AgNPrs was increased, a large decrease in the $I_{\alpha_G}$ and $I_{\alpha_B}$ values were observed, whereas the $I_{\alpha_R}$ values showed a slight decrease. The scattering image at 1 ppm of AgNPs (Fig. 2A), observed by naked eyes and recorded by the camera, shows a green-bluish color with $I_{\alpha_G}$ and $I_{\alpha_B}$ of 58 and 53, respectively. When the concentration of AgNPrs increased, the $I_t$ values of green and blue colors slightly decreased, whereas that of red color increased. The color of the scattering image changed from green-bluish to orange, enabling us to further evaluate the chromatic value of $I_t$. Figure 2C shows the $I_t$ values of each color of AgNPs compared with LSPR peak at 500 nm, at AgNPrs concentrations ranging from 1 to 20 ppm. Interestingly, the calculated $I_{\alpha_G}$ and $I_{\alpha_B}$ values increased with increasing AgNPrs concentration, whereas $I_{\alpha_R}$ was not as sensitive to the increment of the AgNPrs concentration. We could observe color even from the scattering image due to the strong scattering phenomenon of AgNPrs. With our setup, we were capable of capturing pictures even under a low light intensity, with minimum noise. The main aspects guaranteeing the success of our setup are the facilitated path to light through the sample and the high quality of the camera and application used. These also enabled us to further develop a colorimetric detection system using the camera as a detector. Since the $I_t$ value comes from the combined effect of both transmission and scattering phenomena of metal nanoparticles, monitoring such parameter is very useful for studying the change of the optical behavior of metal nanoparticles, thus suggesting a high potential of its use for optical sensors applications. We also investigated blue AgNPs with a maximum LSPR of around 635 nm. In the case of blue AgNPs, the calculated $I_{\alpha_R}$ values were the most sensitive to the AgNPs concentration. The transmitted and scattering images along with their extracted values, including the calculated $I_t$ values, as a function of AgNPs concentration are shown as Supporting Information (see Fig. S2).

Figure 3A shows LSPR spectra and the photographic images of AgNPs (a) and AgNPs after exposure to 80 μM H$_2$O$_2$ for 60 min (b). After AgNPs were exposed to H$_2$O$_2$, the LSPR peak shifted from 500 nm (black solid line) to 460 nm (red solid line), along with a three-fold decrease in the signal intensity. The color of AgNPs, easily observed by naked eyes, changes from red to orange. TEM images in Fig. 3B clearly shows a decrease in the average diameter size of AgNPrs from 50 to 30 nm. Several papers report that AgNPs could be oxidatively disintegrated by H$_2$O$_2$ to silver ion, accompanied by a gradual...
The peak at 500 nm, assigned to the in-plane dipole plasmon band of AgNPrs, shifts toward shorter wavelengths due to the presence of H$_2$O$_2$, as shown in Fig. 3A, and is expected to be due to oxidative etching of metallic silver by H$_2$O$_2$. To further evaluate the alteration of AgNPrs, optical features due to H$_2$O$_2$, essays using solutions with different concentrations, ranging from 10 to 80 $\mu$M, were conducted.

The shift of peak maxima, as representing the change of the optical characteristics of LSPR-based hydrogen peroxide sensor, is shown as a function of time in Fig. 4A. A drastic decrease in the LSPR signal intensity was observed within the initial 60 min of the incubation time for all concentrations tested, suggesting such a period of incubation to be optimal for further investigations over the impact of H$_2$O$_2$ on the optical features of AgNPrs. Figure 4B shows the LSPR spectra of AgNPrs after reacting with H$_2$O$_2$, for 60 min, at concentrations of 10 - 80 $\mu$M. Here, the decrease in the LSPR peak height corresponds to the added amount of H$_2$O$_2$. This observable change in the physical parameter may serve as a way to quantify the amount of AgNPrs oxidized to silver ions in the presence of H$_2$O$_2$. When the H$_2$O$_2$ concentration increases, the red color in transmitted images turn colorless, whereas the scattering images show slight changes in colors from orange to green (Fig. 5A). The $\Delta$I$_{A_G}$ value increased with the increment of the H$_2$O$_2$ concentration, ranging from 10 to 80 $\mu$M, with $R^2 = 0.9792$. H$_2$O$_2$ concentration could be determined with comparable accuracy and precision to the results obtained from the spectroscopic analysis (Fig. 4B). The developed technique represents a simple, rapid, and inexpensive quantitative detection method that makes use of a highly portable instrumental setup. This study also offers an alternative way to use the color information extracted from digital images, under light-controlled conditions, and recorded with a mobile phone camera, in order to construct a quantitative model to determine the analyte concentrations, in our case, H$_2$O$_2$ as a
The developed technique was further utilized for the quantitative determination of glucose. The disintegration of AgNPs and corresponding solution colors of AgNPs resulted from the oxidation reaction with H$_2$O$_2$, generated from the enzymatic oxidation of glucose (D-glucose + O$_2$ + H$_2$O + glucose oxidase enzyme $\rightarrow$ D-gluconic acid + H$_2$O$_2$). A series of glucose solutions with concentrations ranging from 20 to 100 μM was added to the homogenous AgNPs solution, mixed with glucose oxidase enzyme and incubated for 12 h. The transmitted and scattering images and the plot of $\Delta I_{A-G}$ as a function of glucose concentration are shown in Fig. 6. When the concentration of glucose was increased, the transmitted images slightly turned from red to colorless, whereas scattering images showed a color change from orange to green, as shown in Fig. 6A. The $\Delta I_{A-G}$ parameter increases when the concentration of glucose is increased, as seen in Fig. 6B. A linear relationship between $\Delta I_{A-G}$ values and glucose concentration was observed within the range 20 - 100 μM with $R^2 = 0.9869$. The LOD (calculated from 3.3 × standard deviation/slope of regression line) of this technique for the detection of glucose was 19.8 μM. The glucose concentration could be determined with comparable accuracy and precision to the results obtained spectrophotometrically (shown as Supporting Information, Fig. S3). These results imply that glucose could be determined at a micro-molar level using our developed chromatic analysis approach.

**Conclusions**

A 3D-printed device for colorimetric determination of AgNPs was successfully developed and used for the quantitative analysis of AgNPs concentration. Since AgNPs disintegrates in the presence of H$_2$O$_2$, this technique was utilized to quantitatively determine the concentrations of H$_2$O$_2$ and glucose, using glucose oxidase. The $\Delta I_{A-G}$ values increased with increasing H$_2$O$_2$ concentration. Linear relationships between $\Delta I_{A-G}$ and H$_2$O$_2$ were found in the range 10 - 80 μM and between $\Delta I_{A-G}$ and glucose for the range 20 - 100 μM. The developed technique was demonstrated to be a simple, rapid, and reliable detection method without the need of any spectrophotometer. Additionally, the experimental setup is portable and inexpensive. Therefore, we believe that the concept and principle of the proposed colorimetric analysis are robust for biochemical analysis and clinical diagnosis as well as for household biosensors applications.

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**Supporting Information**

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
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