

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_A). The I_A 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 linearities between ΔI_A 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

(Received September 11, 2018; Accepted October 17, 2018; Advance Publication Released Online by J-STAGE October 26, 2018)

Introduction

The unique optical property of metal nanoparticles, called localized surface plasmon resonance (LSPR), is based on the coupling between an external electromagnetic field and a collective electron oscillation in the conduction band of metal nanoparticles.^{1–10} The resulting local electric field is very sensitive in terms of a dielectric function, *i.e.*, any eventual environment alteration leads to detectable changes in their optical properties.^{1,2,6–8} Colloidal solutions of silver and gold nanoparticles, for instance, have unique optical characteristics in the visible light region that enables their LSPR properties alteration to be monitored using UV-visible spectrometry and cameras.^{10–17} Colorimetric detection, benefiting from LSPR phenomenon of plasmonic nanomaterials, are being utilized for biosensor applications.^{10–21} For example, Sittiwong and Unob utilized a colorimetric approach and spectroscopic analysis for the determination of urinary creatinine.¹² The creatinine in human urine was extracted by solid-phase extraction for the elimination of interfering effects from the urine matrix on

creatinine detection. The color of gold nanoparticles (AuNPs), observed by naked eyes, changed due to an aggregation of AuNPs because of creatinine. The quantitative information was successfully accessed by spectroscopic analysis. A colorimetric method *via* image color analysis for detection of hydrogen peroxide (H_2O_2) using silver nanoprisms (AgNPrs) was reported by Nitinaivini *et al.*¹⁹ This work has proposed a strategy for H_2O_2 quantification based on a combination of digital imaging and chromatic analysis of AgNPrs color change. A good linear relationship of the red chromaticity level and H_2O_2 concentration was observed in the range of 10 – 80 μM with $R^2 = 0.9895$, and a limit of detection (LOD) of 6.19 μM was determined.

Smartphones play a pivotal role in everyday life, especially in healthcare monitoring.^{22,23} Adapting a smartphone for chemical detection and analysis is quite a challenge.^{14–17,22–35} Several publications have successfully demonstrated the adaption of smartphones for monitoring biomarkers in biological fluids, such as blood, sweat and oral fluid using enzymatic reactions, paper-based immunoassays, and lateral flow immunoassays.^{30–32,35} Smartphones with a high-performance camera could be utilized for the colorimetric detection of infectious pathogens in the environment and in some clinical samples,³³ and also for monitoring fluorescent signals.^{34,35} For example, Roda *et al.* developed a 3D-printed device for a smartphone-based chemiluminescence biosensor for lactate in oral fluid and

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sweat.²³ This device was able to detect lactate in oral fluid and sweat with a detection limit of 0.5 and 0.1 mmol/L in 5 min, respectively.

In the present work, we propose an image-acquisition technique using a combination of transmitted and scattering light. The developed measurement technique could be carried out by separately recording transmitted and scattering images of red AgNPrs solution, using a mobile phone camera prior to calculating an empirical absorption value (I_A). AgNPrs have a strong LSPR extinction band in the visible light region, called in-plane dipole plasmon band. This band is sensitive to the average diameter size of AgNPrs.^{9,19} When the diameter size of AgNPrs becomes smaller *via* chemical etching, a blue shift of the LSPR peak is observed. Since halide ions and H_2O_2 could etch AgNPrs, quantitative determinations of those chemicals were investigated.^{9,19,36} We are interested in H_2O_2 detection because it is broadly used in industrial processes, such as water treatment, electrical circuit cleaning, and medical purposes.^{19,37} Moreover, for some biomolecules, such as glucose and cholesterol, which are the major chemicals to act as the indicators for medical diagnosis, H_2O_2 is one of products from the enzymatic reaction when they are oxidized by their oxidase enzymes.^{10,19,38,39} Since the calculated I_A value was sensitive to the concentration of AgNPrs, employing H_2O_2 etching and correspondent oxidase enzyme reactions, we used this value to quantitatively determine the H_2O_2 and glucose concentrations. The camera-based detection system developed, based on the optical characteristic of AgNPrs, 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_2O_2) was purchased from Kanto Chemical Co., Inc. Deionized (DI) water was used in the preparation of all solutions. The AgNPrs for colorimetric investigation were prepared following our previously developed methodology (with minor modifications for the sake of simplicity).⁹ 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 $\times$ 10 mm width $\times$ 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

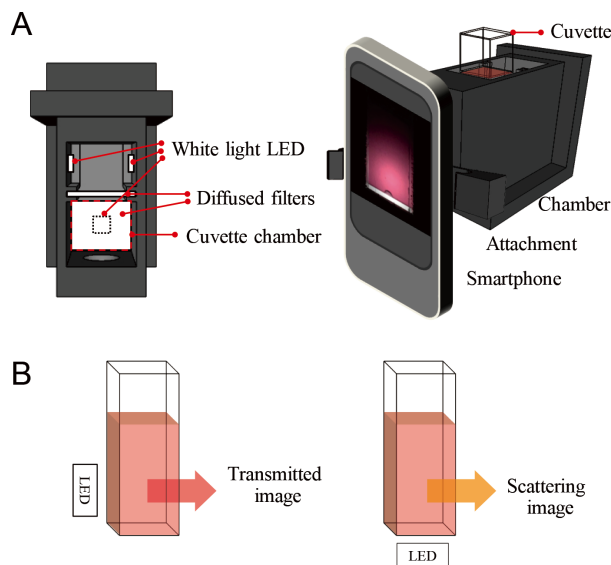


Fig. 1 (A) Printed homemade device for recording transmitted and scattering images of AgNPrs. (B) Schematic of transmitted and scattering images acquisitions.

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 $\times$ 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_{T-R}), green (I_{T-G}), and blue (I_{T-B}) 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_{S-R}), green (I_{S-G}), and blue (I_{S-B}) scattering intensities, respectively. The calculated absorption intensity (I_A), computed by means of an empirical formula, was then calculated as

$$I_A = -\log_{10} \left(\frac{I_T + I_S}{I_0} \right).$$

I_0 was obtained by measuring DI water through the transmission light path.⁴⁰

H_2O_2 sensing by the AgNPrs decomposition profile

Red AgNPrs (20 ppm) solutions with the maximum LSPR excitation at 500 nm were mixed with various concentrations of H_2O_2 at a volume ratio of 1:1 (v/v). The mixed solutions were transferred to a cuvette to monitor the decomposition profile of AgNPrs for 1 – 120 min of the incubation time. The relationship between I_{A-G} of AgNPrs and the H_2O_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 AgNPs on glucose sensing, 1 mL of 20 ppm AgNPs 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_{A-G} of AgNPs 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 AgNPs obtained comparatively from a conventional spectrometer and from our developed chamber using a LED light source. Using a conventional spectrometer, the LSPR spectrum of AgNPs could detect concentrations of 1 – 20 ppm. The corresponding LSPR spectrum and calibration plot within the same concentration range of AgNPs 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 AgNPs are shown in the top row of Fig. 2A. Transmitted images of AgNPs at 1 ppm were colorless. A weak red color was observed at 5 ppm of AgNPs. The red color of AgNPs was stronger when the concentration of AgNPs 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 AgNPs at low concentrations showed high chromaticity levels since the images showed a white background of the diffused filter. When the concentration of AgNPs was increased, a large decrease in the I_{T-G} and I_{T-B} values were observed, whereas the I_{T-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_{S-G} and I_{S-B} of 58 and 53, respectively. When the concentration of AgNPs increased, the I_S 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_A . Figure 2C shows the I_A values of each color of AgNPs compared with LSPR peak at 500 nm, at AgNPs concentrations ranging from 1 to 20 ppm. Interestingly, the calculated I_{A-G} and I_{A-B} values increased with increasing AgNPs concentration, whereas I_{A-R} was not as sensitive to the increment of the AgNPs concentration. We could observe color even from the scattering image due to the strong scattering phenomenon of AgNPs. 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_A 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

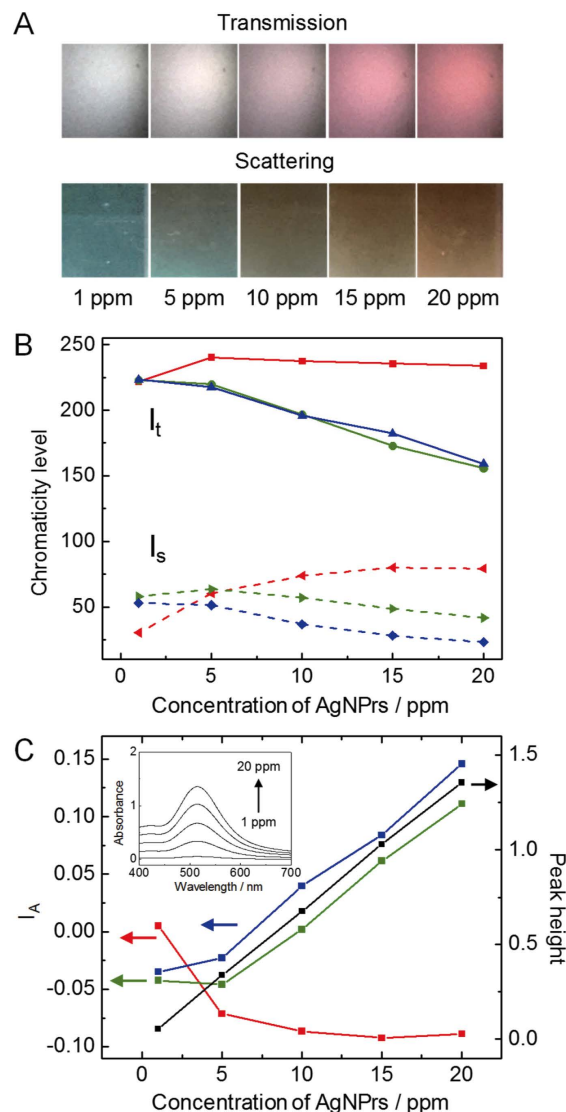


Fig. 2 (A) Transmitted (top) and scattering (bottom) images and their (B) I_T and I_S values of AgNPs. Red, green, and blue chromatic values are shown in red, green, and blue lines, respectively. Solid lines are I_T and dashed lines are I_S . (C) Correspondent peak heights for the calculated I_A values of each color of AgNPs are shown. The concentrations of AgNPs in these experiments range from 1 to 20 ppm.

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_{A-R} values were the most sensitive to the AgNPs concentration. The transmitted and scattering images along with their extracted values, including the calculated I_A 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_2O_2 for 60 min (b). After AgNPs were exposed to H_2O_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 AgNPs from 50 to 30 nm. Several papers report that AgNPs could be oxidatively disintegrated by H_2O_2 to silver ion, accompanied by a gradual

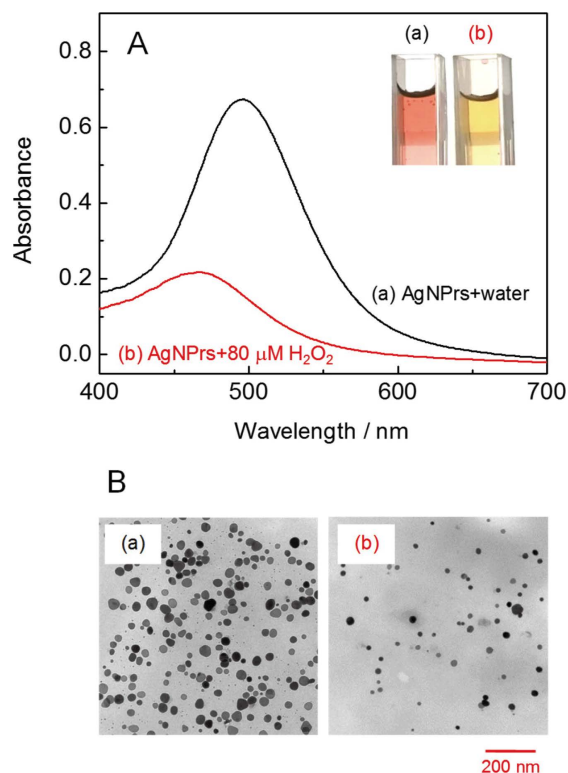


Fig. 3 (A) LSPR spectra and (B) TEM images for the H_2O_2 -free AgNPs solution samples (a) and AgNPs after incubation with 80 μM of H_2O_2 for 60 min (b). Insets show their corresponding photographic images.

color change of the solution from yellow to colorless.^{41,42} The peak at 500 nm, assigned to the in-plane dipole plasmon band of AgNPs, shifts toward shorter wavelengths due to the presence of H_2O_2 , as shown in Fig. 3A, and is expected to be due to oxidative etching of metallic silver by H_2O_2 .^{10,18,19,43,44} To further evaluate the alteration of AgNPs, optical features due to H_2O_2 , essays using solutions with different concentrations, ranging from 10 to 80 μM , 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_2O_2 on the optical features of AgNPs. Figure 4B shows the LSPR spectra of AgNPs after reacting with H_2O_2 , for 60 min, at concentrations of 10 – 80 μM . Here, the decrease in the LSPR peak height corresponds to the added amount of H_2O_2 . This observable change in the physical parameter may serve as a way to quantify the amount of AgNPs oxidized to silver ions in the presence of H_2O_2 , once a proper calibration plot is given. The H_2O_2 calibration plot within this concentration range is shown by the inset of Fig. 4B. In addition, a blue shift of the LSPR spectrum was observed, indicating that the average diameter size of the nanoparticles was decreased. When the external electromagnetic field couples with the oscillated electron with higher energy (small size of AgNPs), the LSPR peak at a shorter wavelength is observed. Herein, the average size of AgNPs was decreased by H_2O_2 etching, so the LSPR peak then shifted to shorter wavelength. At 80 μM of H_2O_2 , where the largest shift of LSPR peak was

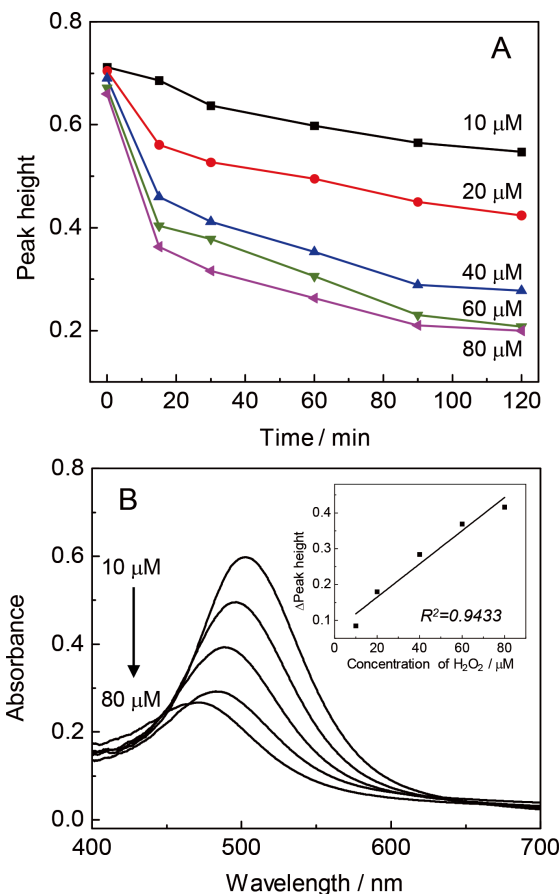


Fig. 4 (A) Relationship between the AgNPs LSPR signal intensity and the incubation time at various H_2O_2 concentrations for evaluating the optimal incubation time for further analysis. (B) Extinction spectra of AgNPs when exposed to H_2O_2 at various concentrations from 10 to 80 μM , for 60 min. The inset shows a plot of the Δ peak height versus the H_2O_2 concentration.

observed, AgNPs solution sample was dominated by the decrement in average diameter size. The concentration of 80 μM of H_2O_2 was also the upper limit of detection of the present developed technique.

Since a significant changes of the AgNPs optical features occur after the oxidation reaction in the presence of H_2O_2 , we proposed a colorimetric approach utilizing RGB image capturing for quantitative analysis. Our developed technique was tested for investigating the H_2O_2 concentration utilizing the calculated value of I_A . When the H_2O_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 ΔI_{A-G} value increased with the increment of the H_2O_2 concentration (Fig. 5B). We observed a linear relationship between the ΔI_{A-G} value and the H_2O_2 concentration, ranging from 10 to 80 μM , with $R^2 = 0.9792$. H_2O_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_2O_2 as a

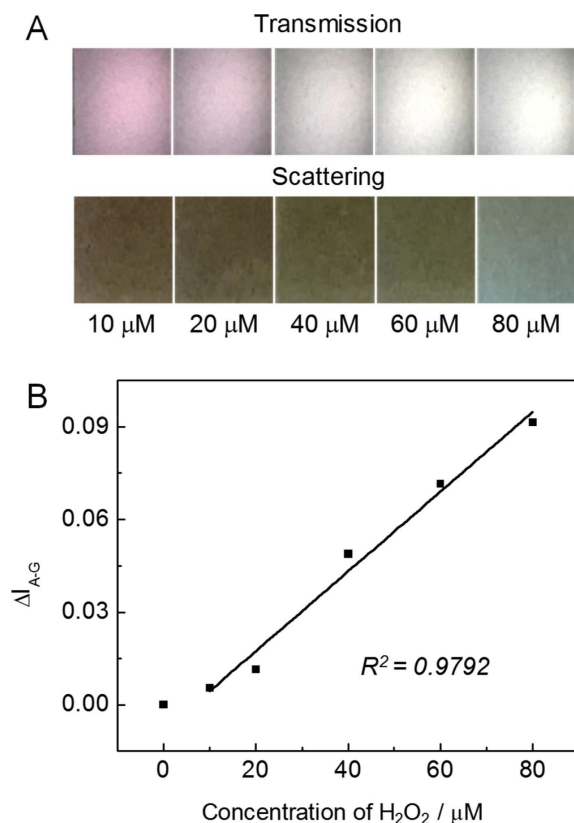


Fig. 5 (A) Transmitted and scattering images of AgNPs solutions after incubation with H₂O₂ at various concentrations from 10 to 80 μM for 60 min. (B) Plot of the ΔI_{A-G} value as a function of the H₂O₂ concentration.

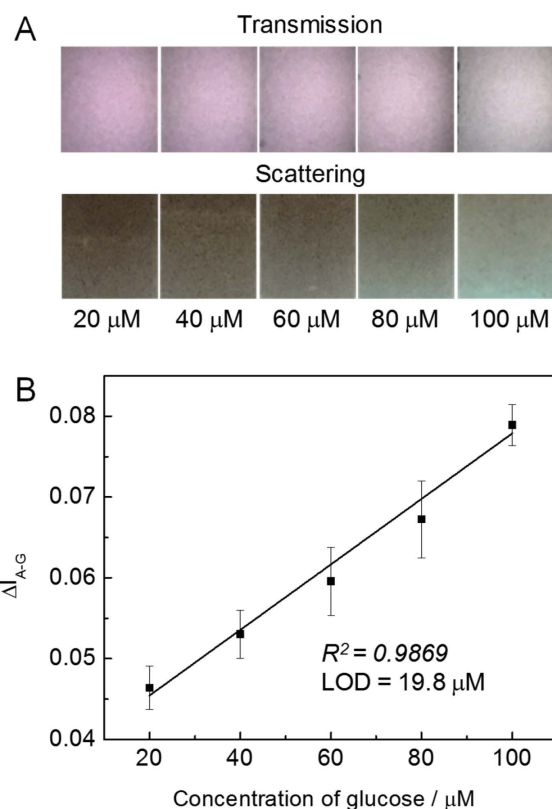


Fig. 6 (A) Transmitted and scattering images of AgNPs solutions after incubation with glucose at various concentration from 20 to 100 μM for 12 h. (B) Plot of ΔI_{A-G} value as a function of glucose concentration.

typical model.

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 H₂O₂, generated from the enzymatic oxidation of glucose (D-glucose + O₂ + H₂O + glucose oxidase enzyme → D-gluconic acid + H₂O₂). 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 ΔI_{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 ΔI_{A-G} parameter increases when the concentration of glucose is increased, as seen in Fig. 6B. A linear relationship between ΔI_{A-G} values and glucose concentration was observed within the range 20 – 100 μM with $R^2 = 0.9869$. The LOD (calculated from $3.3 \times \text{standard deviation/slope of regression line}$) 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 spectroscopically (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 H₂O₂, this technique was utilized to quantitatively determine the concentrations of H₂O₂ and glucose, using glucose oxidase. The ΔI_{A-G} values increased with increasing H₂O₂ concentration. Linear relationships between ΔI_{A-G} and H₂O₂ were found in the range 10 – 80 μM and between ΔI_{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.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP16K13662 and JP17H03231, and Niigata University U-go grant.

Supporting Information

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

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