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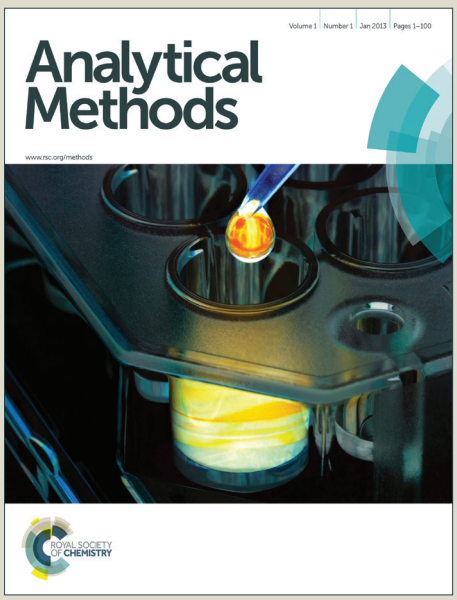

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ARTICLE TYPE

Colorimetric Determination of Hydrogen Peroxide by Morphological Decomposition of Silver Nanoprisms Coupled With Chromaticity Analysis

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A novel colorimetric method with image colour analysis for highly sensitive and accurate detection of hydrogen peroxide using starch-stabilized silver nanoprisms (AgNPrs) is proposed. AgNPrs were morphologically decomposed by a low concentration of hydrogen peroxide revealed by UV-visible absorption spectroscopy and transmission electron microscopy (TEM). The morphological changes of AgNPrs leaded to an appreciable colour change of AgNPr solution from red to orange, and finally yellow, respectively. A good linear relationship between the wavelength shift of AgNPrs and the H_2O_2 concentration can be obtained. The solution phase detection of H_2O_2 by the direct morphological change can be accomplished without any surface modification of AgNPrs. In addition to the conventional determination of H_2O_2 concentration utilizing spectroscopic data, a new and simple colorimetric strategy based on the chromaticity analysis of AgNPr solution was demonstrated. The strategy can be employed not only for visual detection of H_2O_2 by naked eye but also for reliable and convenient method in quantification of H_2O_2. Hydrogen peroxide concentration at 1.57 μM can be recognized by naked-eye observation with good accuracy, stability and reproducibility. Furthermore, the proposed protocol can be applied to determine a glucose concentration through glucose-oxidase system. A good linearity between the red chromaticity of solution colour and the glucose concentrations was observed. The new colorimetric determination of hydrogen peroxide utilizing the digital image analysis on colour changes from AgNPr shape decomposition will open up an alternative method of simple, rapid and reliable detection of hydrogen peroxide and can realize its future applications in biochemical analysis or clinical diagnosis.

Introduction

Metal nanoparticles (MNPs) have stimulated a great deal of research interest due to their shape- and size-dependent optical, electrical and chemical properties[1-3]. The well-known optical property of MNPs, called "localized surface plasmon resonance (LSPR)", is based on the coupling between an external electromagnetic field and the collective electron oscillations in the conduction band of the metal. The shape and size of MNPs present in a dielectric environment can induce different collective oscillations of electrons on MNPs surfaces. In the case of silver and gold nanoparticles, this unique nano-associated property is strongly related to the colour of the colloidal MNP solution observed in the UV-visible region which can be employed in various applications as visual sensors[4-6].

Colormietric approaches have extensively grown in chemical sensing applications due to its simplicity in detection by the naked eye. With the recent advancement of nanotechnology, designing colorimetric sensors by MNPs is a promising field which is showing increasing interest, especially for gold and silver nanoparticles, which contain a strong plasmonic signal. This allows these metal nanoparticles to be utilized as an ideal chromatic agent for colorimetric sensor design. The aggregation phenomenon is a good example of how to utilize the colorimetric method in the design of a colorimetric sensor[7-9]. Colormietric methods based on the basis of particles' aggregation have strong advantages in terms of their simplicity and rapidity. However, there are some critical drawbacks: low accuracy, poor stability, and irreproducibility. Therefore, most of the colorimetric sensors based on plasmonic nanoparticle aggregation might not be appropriate for the quantitative detection of an unknown sample[10-12]. To avoid the drawbacks of the aggregation approach, colorimetric detection methods based on the morphological transformation of nanoparticles have recently been developed[13-15]. There are several advantages of this strategy. First of all, surface modification is not required in order to induce an aggregation process. In addition, nanoparticles are well-dispersed during the shape transformation process resulting in a stable solution colour and reliable UV-visible extinction band which is unambiguous, essential for its employment in quantitative analysis.

Silver nanoprisms (AgNPrs) have a strong LSPR extinction band which can be tuneable throughout the visible and near-infrared (NIR) regions by controlling their edge length, thickness, aspect ratio, and morphology[16,17]. The particle shape of AgNPrs is found to be sensitive to chemical molecules, metals and ionic species[13,15,17]. Specifically, the etching phenomenon of AgNPrs has been thoroughly studied and employed to determine and control particle morphology in nanomaterial synthesis. Furthermore, this phenomenon can be used for analytical purposes. The interaction between AgNPrs and anions has been studied by many researchers[18-20]. It has been found that halide

ions, e.g. Cl^-, I^- and Br^-, could etch the side faces of the AgNPrs, resulting in the transformation of the structure into a smaller disk-like shape. Huang's group[21] studied the time-dependent surface plasmon resonance spectroscopy of AgNPrs in the presence of halide ions and introduced that this interaction will induce the shift of the SPR band of AgNPrs. Therefore, a simple sensing method to detect inorganic anions by SPR shifts of AgNPrs was demonstrated.

Hydrogen peroxide (H_2O_2) is broadly used in either the industrial processes, such as water treatment and electric circuit cleaning, or in medical purposes like wound cleaning. The accurate determination of H_2O_2 concentration is important as high concentrations of H_2O_2 could induce cellular damages. Moreover, some target analytes, e.g. glucose and cholesterol, can give H_2O_2 as a major product using the oxidase enzyme. This chemical mechanism offers an opportunity to indirectly quantify these target analytes by determination of the H_2O_2 produced though the oxidase chemical reaction. Conventionally, H_2O_2 determination was based on several analytical techniques, such as spectrophotometry, voltammetry, chemiluminescence and electrometric analysis. However, these instrumental techniques are expensive, complicated and not portable[22-24]. Therefore, a simple, rapid, cheap, and also portable technique for H_2O_2 determination is preferred. Many researchers, for example, Filippo et.al.[25] performed the experiment using silver nanospheres (AgNSs) as a hydrogen peroxide sensor. From this research, the intensity of LSPR showed a good linear response with varying concentrations of H_2O_2, indicating the possibility of applying AgNSs as a hydrogen peroxide sensor. Yunsheng Xia et al.[14] investigated the application of silver nanoprisms (AgNPrs) as a colorimetric sensor for glucose. When various concentrations of glucose were added to a mixture of AgNPrs and glucose oxidase, the colour of the solution changed corresponding to the concentration of glucose added, thus, making the AgNPrs applicable for glucose sensing using the colour of the colloidal solution. Recently in our research group, we have developed the solution-phased chemical fabrication of starch-stabilized AgNPrs with controllable longitudinal LSPR wavelengths by H_2O_2-induced shape transformation of AgNSs[26]. AgNPrs with various vivid colours in the visible region, e.g. red, pink, violet, blue, and light blue can be selectively fabricated with high stability. The ability to control the lateral length, and therefore the in-plane dipole plasmon resonance of AgNPrs, allows us to fully investigate the decomposition phenomenon of AgNPrs of various colours with H_2O_2 and select the most suitable AgNPrs for developing as a colorimetric hydrogen peroxide sensor. In contrast to other reported research on applying AgNPrs as colorimetric sensors, our AgNPrs were stabilized by soluble starch, a biopolymer, instead of conventional capping molecules i.e. citrate. Therefore, it is also interesting to study the similarity and difference of the chemical and morphological decomposition profiles of starch-stabilized AgNPrs by H_2O_2 in comparison with those reported in the literature. The vivid, clear, and contrastable colour change of starch-stabilized AgNPrs when exposed to H_2O_2 enables the development of a naked-eyed colorimetric sensor and a portable sensor utilizing digital image analysis. In addition, starch is an environmentally benign and nontoxic biopolymer. Therefore, our starch-stabilized AgNPrs offer an alternative way to be used in biological and biochemical studies.

In this study, the decomposition mechanism of starch-stabilized AgNPrs on the interaction with H_2O_2 was intensively investigated. The decomposition phenomenon was mainly monitored by the shift of the in-plane dipole and out-of-plane quadrupole LSPR bands, which are related to the lateral size and aspect ratio of the AgNPrs, respectively. The combination of the variations in the LSPR bands and TEM micrographs can be used to reveal the change in morphology of AgNPrs when exposed to H_2O_2. In our protocol, a surface modification of AgNPrs with additional surfactants and capping molecules is not required. Additionally, the change in LSPR bands are directly correlated to the change of AgNPrs structures, not indirectly conveyed through an aggregation process. The direct morphological response of AgNPrs with the target analyte will reduce the complexity of producing and employing AgNPrs as a colorimetric sensor. Insight understanding of the decomposition mechanism can lead to the development of a H_2O_2 determination protocol by using the change in LSPR and the solution colour of AgNPrs to quantify a H_2O_2 concentration. In this paper, we also develop an alternative protocol using digital image analysis to directly quantify the amount of H_2O_2 from the changes of the AgNPrs' solution colour. Moreover, we also demonstrate a simple but effective strategy for the determination of glucose at the submicromolar level, using unmodified AgNPrs and a colorimetric approach with theoretical simplicity and low technical demands.

Experimental

Reagents and chemicals

Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), soluble starch, and H_2O_2 (30% w/w H_2O_2) were purchased from Merck. All chemicals were analytical reagent (AR) grade and were used as received. Glucose oxidase enzyme was obtained from Sigma-Aldrich and stored in a freezer at 4 ^oC to preserve the enzyme activity. All solutions were prepared with de-ionized (DI) water. All glassware and magnetic stirrer bars were rinsed with 2 M of nitric acid, cleaned with detergent, and finally rinsed with copious amount of DI water prior to use in the experiment.

Preparation of silver nanoprisms (AgNPrs)

AgNPrs were fabricated via a H_2O_2-triggered shape transformation reaction of silver nanospheres (AgNSs) as reported in our previous publications[26,27]. Briefly, silver nanospheres (AgNSs) were synthesized by the chemical reduction of $AgNO_3$ (3.71×10^{-3} M, 500 mL) with $NaBH_4$ (5.76×10^{-3} M, 500 mL) utilizing 2% (w/v) soluble starch as a stabilizer. The excess $NaBH_4$ was eliminated by gently heating the colloidal AgNS solution for 2 hours. After the colloidal solution was cooled down to room temperature, the total volume of solution was adjusted to 1000 mL. The obtained colloidal AgNS solution appeared as a dark yellow-brown solution.

The shape transformation of AgNSs to AgNPrs was carried out by feeding 30% wt. H_2O_2 into the colloidal AgNS solution. Typically, 0.158 mL of 30% wt. H_2O_2 was injected into 50 mL of the colloidal AgNS solution at a rate of 10.09 mL/min, under vigorous stirring by a high speed disperser (IKA® T25 ULTRA-TURRAX®). The volume and injection rate of H_2O_2 were controlled by a syringe pump (NE-1000 Programmable Single

Syringe Pump, New Era Pump System, Inc.). The solution gradually changed its colour from yellow to red wine at a mole ratio of H_2O_2:AgNSs equal 15:1, indicating the complete formation of AgNPrs. The synthesized AgNSs and AgNPrs were characterized by UV-visible absorption spectroscopy (Ocean Optics USB4000 fiber optic spectrometer coupled with a DH-2000 deuterium/halogen light source) and viewing with a transmission electron microscope (TEM High-Resolution Electron Microscope EM10A/B ZEISS Germany). Dimensions of the silver nanoparticles were measured directly from the TEM micrographs using ImageJ software (a Java program developed by the National Institute of Mental Health[28]). The longest lateral length AgNPrs or largest diameters of AgNSs were measured for at least 300 particles in order to construct the lateral size or particle size distribution histogram, respectively.

H_2O_2 sensing by decomposition profiles of AgNPrs

Red AgNPrs (20 ppm), with maximum LSPR extinction at 500 nm, were mixed with various concentrations of H_2O_2 at volume ratio of 1:1 (v/v). The mixed solution was transferred to a 1 cm quartz cuvette, in order to monitor the decomposition profile of AgNPrs with UV-visible spectroscopy from 1-120 minutes of incubation time at room temperature. The relationship between the wavelength shift of the in-plane plasmon resonance band ($\Delta\lambda = \lambda_{max}$ of the original AgNPrs – λ_{max} of decomposed AgNPrs) and the incubation time was monitored to follow the decomposition rate of the reaction. In addition, the solution colour at various incubation times was recorded and investigated in order to facilitate the development of the method as a potential naked eye sensor for quantifying H_2O_2 concentrations.

Experimental Protocol for glucose sensing

D-glucose was purchased from Carlo Erba. A standard solution of D-glucose (5.55×10^{-3} M) was prepared by dissolving 0.01 g of D-glucose in 10 mL of DI water. The D-glucose solutions with concentrations ranging from 20 to 100 µM were prepared by the serial dilution of the stock solution with DI water. To investigate the performance of AgNPrs on glucose sensing, 1 mL of 10 ppm AgNPrs was mixed with 20 µL of glucose oxidase enzyme (10.0 mg L^{-1}) and incubated for 5 minutes. Then, 1 mL of the prepared D-glucose solution was added to the mixture and incubated for 60 minutes. The absorbance of the solutions was measured at room temperature. In a control experiment, the homogeneous system containing only AgNPrs and glucose oxidase enzyme was investigated under the identical conditions excluding the added glucose.

Results and Discussion

The extinction spectra, TEM micrographs, and the photographic images of the silver nanospheres (AgNSs) and silver nanoprisms (AgNPrs) are shown in Fig.1. The extinction spectrum of AgNSs exhibits a clear absorbance peak at ~400 nm, which is the characteristic dipole plasmon resonance of spherical silver nanoparticles[29-31]. AgNSs possessed a uniform particle size distribution as observed in TEM micrographs with an average particle size of 7.5 nm. When H_2O_2 was introduced, the band AgNS colloid, the extinction spectrum of AgNPrs exhibited two new distinct plasmon bands, and the peak of the spherical AgNPs

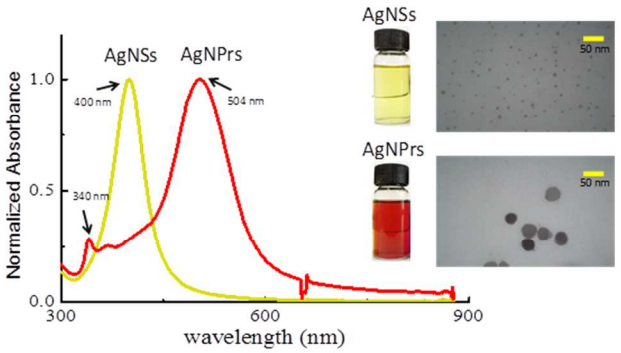

Fig. 1 The UV-visible extinction spectra, TEM micrographs and the photographic images of the solution colour of spherical silver nanoparticle (AgNSs) and the transformed silver nanoprisms (AgNPrs) through the structural evolution induced by H_2O_2

at 400 nm completely vanished. Compared to the spectrum in the benchmark references[26,29-31], the LSPR bands at 340 nm and 504 nm can be assigned to the out-of plane quadrupole plasmon resonance associated with the aspect ratio (lateral size/thickness) of the nanoprisms and the in-plane dipole plasmon resonance correlated to the lateral size of the nanoprisms, respectively. These observations suggest that the AgNSs were completely transformed to the plate-like nanoparticles, as shown in TEM micrographs of Fig. 1. The obtained extinction spectra are in good agreement with the TEM micrographs. The colour of the colloidal solution completely changed from yellow to red wine when a molar ratio of [H_2O_2]:[Ag] equal to 15:1 was introduced. The colloidal AgNPrs particles were synthesized for three repetitions to ensure that the synthesized process was reproducible. The UV-visible spectra of AgNPrs for the three repetitions are shown in ESI† Fig. S1. The similar major LSPR band positions suggest that AgNPrs with uniform size and shape can be reproducibly synthesized though our process. The influences of pH and temperature on the synthesized of AgNPrs were investigated as shown in ESI† Fig. S2

The ability of H_2O_2 to donate oxygen or accept electrons makes it a powerful oxidizer. Silver nanoparticles can be oxidatively disintegrated by H_2O_2 to Ag^+. There are several reports[25, 32, 33] showing that the yellow AgNS solution gradually changed to colourless after interacting with H_2O_2. The detection of the attenuation of the plasmon absorption of AgNSs after the etching reaction by H_2O_2 can only be accomplished with UV-visible spectrophotometer. The attenuation of the solution colour without a colour change when AgNSs interacted with H_2O_2 imposes an unavoidable limitation for utilizing AgNSs as a naked-eye hydrogen peroxide sensor. However, when AgNPrs were exposed to 100 µM H_2O_2 at room temperature, the attenuation of the in-plane dipole plasmon absorption, along with a shift in the peak position, were observed as shown in the time-resolved UV-visible extinction spectra (Fig. S3 in ESI†). This phenomenon is due to the oxidative etching of metallic silver by H_2O_2[14,16,34]. This etching reaction of H_2O_2 induces the degradation of silver atoms on AgNPrs to silver ions (Fig. S4 in ESI†). For an evaluation of the optical characteristics of AgNPrs as an improved LSPR-based hydrogen peroxide sensor, H_2O_2 solution with a concentration ranging from 1 to 1,000 µM was introduced into the prepared AgNPrs dispersion at a volume ratio of 1:1 and placed into the quartz cuvette. The change in its optical characteristics with incubation time (0, 5, 15, 30, 60 and 120 min)

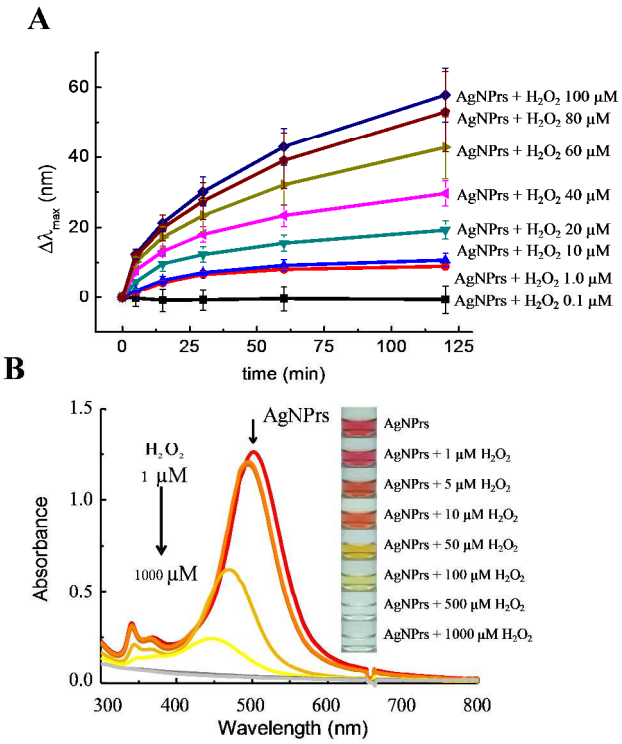

Fig. 2 (A) Relationship between the Δλ of in-plane dipole LSPR peak of AgNPrs and incubation time at various H_2O_2 concentrations for evaluating the optimal incubation time for the further analysis. (B) Extinction spectra of AgNPrs when exposed to H_2O_2 at various concentrations from 1 to 1,000 μM with the inset photographs showing the colour of colloidal AgNPr solution after the incubation with H_2O_2 for 60 min.

was monitored through the in-plane dipole LSPR peak (at ~504 nm) in the extinction spectra. The change of Δλ representing the change of the optical characteristics of the LSPR-based hydrogen peroxide sensor with time is shown in Fig. 2A. A drastic change in Δλ could be observed after 60 min of incubation time. This suggests that the optimal incubation time to investigate the change of the optical characteristic of AgNPr solution interacting with the added H_2O_2 was 60 min. Fig. 2B shows the extinction spectra and the corresponding solution colour of AgNPrs after reacting with H_2O_2 at various concentrations for 60 min. The colour of AgNPr suspension changed noticeably from red to orange and then yellow, respectively, corresponding with the amount of H_2O_2 added. In addition, a blueshift of the in-plane dipole plasmon resonance peak of AgNPrs and a concomitant decrease of the peak intensity were observed. On the other hand, the out-of plane quadrupole plasmon resonance peak at 340 nm was unchanged. These observed phenomena indicate that the lateral size of AgNPrs decreased, but the particle aspect ratio remained the same. Therefore, the thickness of AgNPrs concomitantly decreased along with the lateral size. The spectral change of AgNPrs in the solution is a manifestation of the morphological transformation that occurred with the AgNPrs. We also investigated the possibility of employing blue-AgNPrs with a maximum LSPR band at 590 nm as a hydrogen peroxide sensor. However, the decomposition of blue-AgNPrs by H_2O_2 did not lead to the significant in-plane LSPR wavelength shift, i.e. the solution colour was almost insensitive to the change of H_2O_2

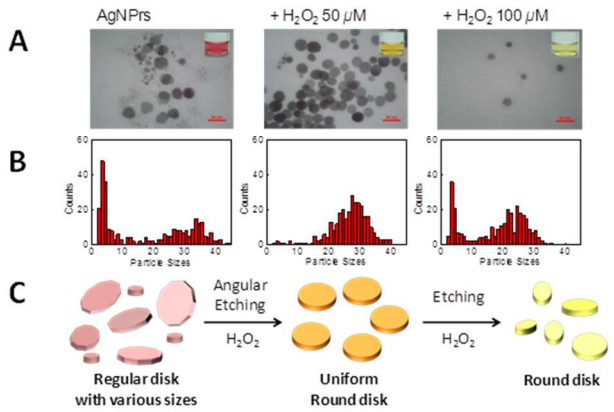

Fig. 3 (A) Representative TEM micrographs and (B) lateral size distribution (300 particles) of AgNPrs before and after incubation with 50 and 100 μM of H_2O_2 for 60 min. (C) The schematic drawing depicts the disintegration of AgNPrs when interacted with H_2O_2 based on the results from TEM micrographs.

concentrations (Fig. S5 in ESI†). Therefore, only red-AgNPrs were selected for further analysis in this paper.

There are several pieces of previous research show that some chemicals, such as halide ions or peroxide ions, have a good affinity to adsorb and disintegrate the silver nanoparticle's surface to other shapes[15-16,18-21]. In general, the sharp corners of silver nanoprisms have a higher chemical activity that can be preferentially disintegrated by other chemical species or under strong conditions (high temperature, high pH etc.). This phenomenon results in a blue-shift of the in-plane dipole LSPR peak[13,18-21]. For our observations, the in-plane LSPR peak of AgNPrs blue-shifted and the intensity also decreased in the presence of H_2O_2. This suggests that the population of the AgNPrs might be etched or destroyed by H_2O_2. To gain an insight into the morphological transformation of AgNPrs, TEM was employed. Fig. 3A shows the TEM micrographs, including the colloidal solution of the initial AgNPrs and the AgNPrs with an orange and yellow colour obtained after the etching process by H_2O_2 at 50 and 100 μM, respectively. The lateral size distribution plots of AgNPrs measured from 300 representative particles are shown in Fig. 3B. The initial red-AgNPrs before interacting with H_2O_2 exhibited regular nanodisks with various sizes, with two distributions of edge lengths with an average size of ~5.5 and 32 nm. When the initial AgNPrs were incubated with H_2O_2, most of the AgNPrs were rounded and transformed into uniform nanodisks with a unimodal lateral length distribution centered at an average size of ~27 nm. When AgNPrs were exposed to H_2O_2 at a concentration of 100 μM, the AgNPrs were still in a disk shape. However, the two distributions of edge lengths with an average size of ~5.5 and 24 nm were observed again. This phenomenon suggests that the AgNPrs randomly disintegrated in every dimension from a larger disk to a smaller disk. The lateral length contraction of AgNPrs could result in a drastic change in the in-plane dipole plasmon resonance position, correlating with a large shift of the colloidal solution colour from red wine to orange and yellow. The extent of the AgNPr decomposition directly correlated with the H_2O_2 concentration. The morphological decomposition of AgNPrs by H_2O_2 etching represents the pathway by which AgNPrs degenerated to smaller nanodisks accompanying silver ion

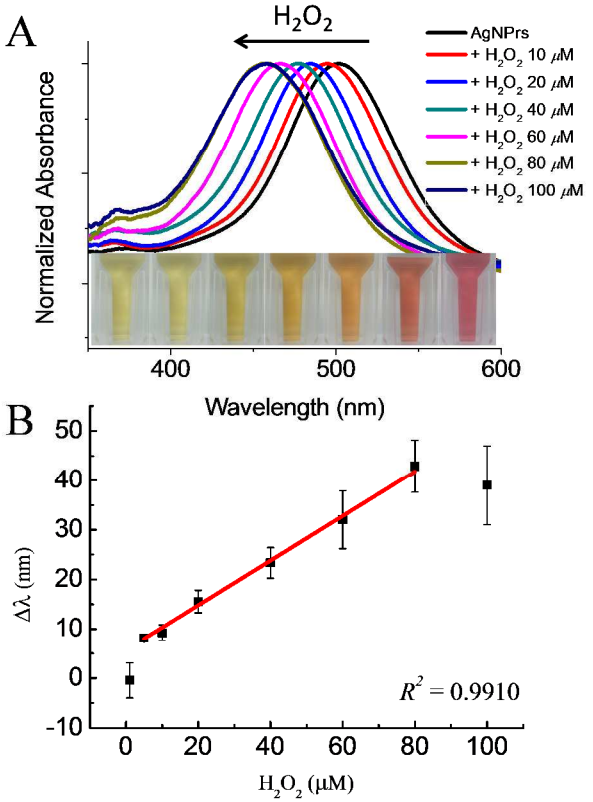

Fig. 4 (A) Normalized LSPR spectra of the AgNPrs after incubation with various concentrations of H_2O_2 from 0 to 100 μM for 60 min. The inset shows digital photographs of the corresponding AgNPr colloidal solutions. (B) Plots of the LSPR peak shift (Δλ) versus H_2O_2 concentrations after performing 3 experiments.

solubilization. This observed phenomenon is different from the previous reports on halide ion etching of AgNPrs. Halide ions, i.e. Cl^-, Br^-, and I^-, can selectively modify the AgNPrs by rounding the tip or edge which finally yields nanodisks or nanospheres[19,35], while H_2O_2 totally disintegrates AgNPrs to Ag^+ without preference of the final shape. A schematic drawing of the possible morphological degeneration mechanism is shown in Fig. 3C.

When AgNPrs were exposed to H_2O_2 at a concentration higher than 100 μM, the AgNPrs were completely disintegrated to silver ions and the colloidal solution became clear and transparent. Therefore, the sensitivity and other performance indices of the proposed H_2O_2 naked-eye sensor were further investigated a H_2O_2 concentration upper limit of 100 μM. A series of H_2O_2 solutions with various concentrations ranging from 10-100 μM were added to the homogenous system of the AgNPr solution and incubated for 60 min. A relationship between the LSPR peak shift (Δλ) calculated from the difference of the in-plane LSPR peak from the original AgNPrs and the transformed AgNPrs, along with the corresponding solution colour, are illustrated in Fig. 4. As shown in Fig. 4A, a gradual blue shift of the in-plane LSPR band and a distinct colour change of the AgNPr solution (red → orange → yellow) can clearly be observed as the H_2O_2 concentration increases. In addition, a good linear relationship between the LSPR peak shift (Δλ) and the concentration of H_2O_2 ranging from

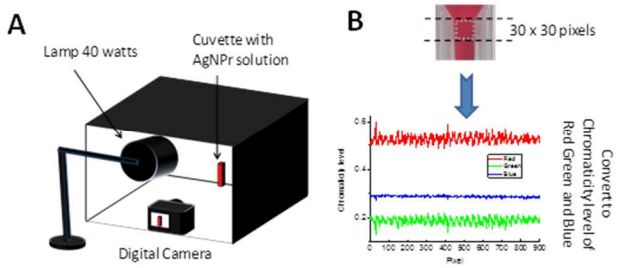

Fig. 5 Chromatic analysis for quantifying H_2O_2 concentration. (A) The imaging studio setup for colormietric determination of H_2O_2 concentration. (B) Digital photograph of the solution colour with cropped area of 30 x 30 pixels (=900 pixels) and the chromaticity level calculated by using the relative intensity of a particular of Red, Green and Blue components in the digital photograph.

10-80 μM with $R^2 = 0.9910$ is observed in Fig. 4B. The limit of detection, LOD (calculated from 3.3 × standard deviation / slope of the regression line) for H_2O_2 was 1.57 μM.

In our system, AgNPrs were stabilized by soluble starch. Starch molecules can be degraded and fragmented to smaller organic acids (e.g. formic acid, acetic acid, etc.) under specific circumstances, such as alkaline treatment and oxidative environments[32,37]. In order to investigate whether soluble starch was chemically interfered with by H_2O_2, i.e. whether H_2O_2 acted as a strong oxidizing agent or not, the molecular characteristics of starch on AgNPr surfaces under various experimental conditions were further investigated by ATR FT-IR spectroscopy (Fig. S6 in ESI†). Starch has a few common ATR FT-IR bands at 1200-900 cm^{-1} and at 930 cm^{-1} representing the bridge (β C1–O–C4) stretching and the skeleton mode vibration of the α-1,4 glycosidic linkage (C-O-C), respectively. From the ATR FT-IR spectra of pure starch, starch-stabilized AgNPrs and starch-stabilized AgNPrs after the addition of H_2O_2, no significant change in these common bands was observed at any concentration of H_2O_2. As reported in a previous publication[38], starch can be oxidatively degraded by H_2O_2 only when divalent metal ions, notably Fe^{2+} or Cu^{2+}, were presented. However, there were only monovalent metal ions (Ag^+) present in our system after the etching reaction of AgNPrs by H_2O_2. Therefore, there was no appropriate catalyst for starch degradation and soluble starch did not interfere with our analysis. These results suggested that H_2O_2 directly interacted only with AgNPrs.

The contrastable colour change of starch-stabilized AgNPrs responding to the interaction with H_2O_2 offers the possibility for developing the AgNPrs as a quantitative naked-eye sensor for H_2O_2. According to the fast development of camera technology for capturing the high-definition photos in the past decade, chemical imaging has currently appeared as an attractive analysis technique. The technique not only resolves the shortcomings of the conventional spectroscopic techniques (e.g. expensive, complicated a difficult instrumental set up, etc.), but also extends the capability to measure huge spectral information in a short analysis time. Since the extent of colour change of starch-stabilized AgNPrs exposed to H_2O_2 is a systematic response to the H_2O_2 concentration, we propose a colorimetric approach utilizing RGB image capturing for the quantitative determination

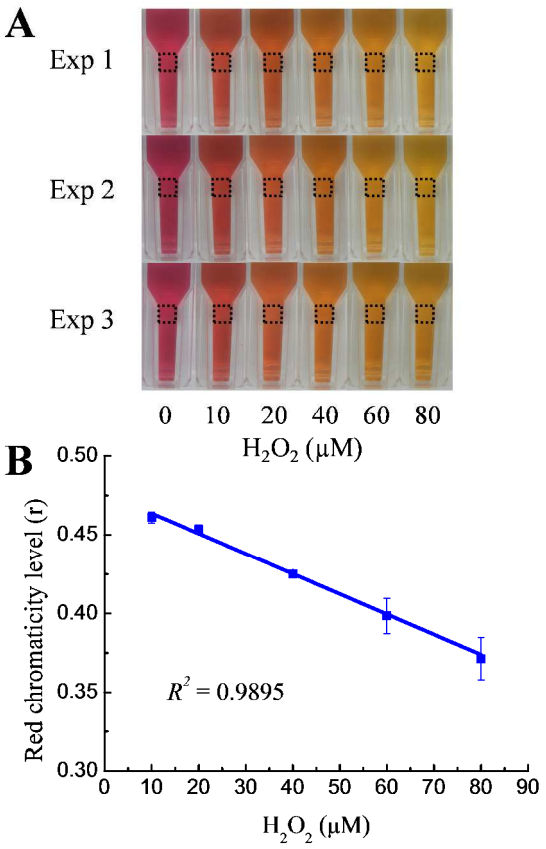

Fig. 6 (A) Digital photographs of the AgNPr solutions after incubation with H_2O_2 at various concentrations from 0 to 80 μM for 60 min. The different colour transitions from red to yellow in the cuvette depending upon the added H_2O_2 concentrations were observed. The square box represents the selected area (30 x 30 pixels) for calculating the red chromaticity level. (B) Plot of the red chromaticity level as a function of H_2O_2 concentration. The error bars represent the standard deviations based on three independent measurements.

of H_2O_2. Red-green-blue (RGB) colour is common spectral information extracted from digital images which can be employed as a variable for quantitative analysis[39-41]. A schematic drawing of the RGB imaging set up is shown in Fig. 5A. The RGB imaging apparatus consisted of a digital camera (FUJIFILM FinePix S5700), a white light source (SYLVANIA Minilynx Economy 20W), and a homemade black box (dimension 21.0 ×.29.7 × 21.0 cm). The focal distance between samples and the digital camera was constantly fixed at 7.0 cm in every experiment. Camera parameters were adjusted as follows: JPEG (highest resolution: 3072 x 2304 pixels) ; ISO 100; white balance was manually set up compared with the standard white colour from a white balance filter (Seculine Prodisk II) to constrain the background colour variation in every snapshot. Excessive ambient light was kept away whilst utilizing the homemade black box. Each captured image consisted of the AgNPr coloured solution in a transparent cuvette (Disposable Cuvettes 1.5 mL Semi-micro PS). The image in the bottleneck area was cropped into the 30 x 30 pixels (=900 pixels) and stored in a JPEG format using Microsoft Paint Software. The cropped image was further processed with MATLAB version R2012a (7.14.0.739) with an image toolbox in order to extract the intensities of the three colour channels, which were red, green and blue of each pixel.

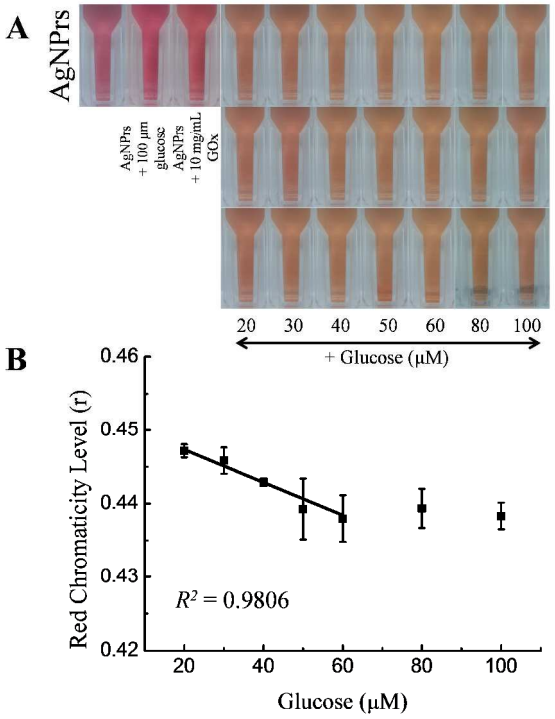

Fig. 7 (A) Digital photographs of the AgNPr solutions after incubation with glucose (20-100 μM) and glucose oxidase enzyme for 60 min. The photographs show the different colour transitions of AgNPrs from red to orange in the cuvette after exposing to glucose at various concentrations and glucose oxidase enzyme. (B) A plot of the red chromaticity level as a function of glucose concentrations. The error bars represent the standard deviations based on three independent measurements.

In our study, only the red channel was selected for further investigation because it provides the strongest intensity that corresponds to the highest sensitivity compared to the other channels.

Chromatic analysis was carried out only on the extracted red channels. The red chromaticity level (r)[39] in each selected pixel was calculated as (r) = R / (R + G + B) where R, G and B are the three primary colour component intensities for the red, green and blue channels, respectively. The sensitivity of the red chromaticity level was further investigated and validated for utilizing as the promising quantitative index for the determination of H_2O_2. A series of H_2O_2 solutions with concentrations ranging from 10-80 μM were added to AgNPrs solutions in transparent cuvettes and incubated for 60 min. The identical experiments were repeated for 3 times in order to observe how the colour of the AgNPrs solution varied when interacting with H_2O_2 as shown in Fig. 6A. Even though the colour change of the AgNPrs solution strongly depends on the amount of H_2O_2 added, there was no significant difference in the solution colour between the three replicates (Fig. 6A). These results suggested that our approach was reproducible. The digital colorimetric analysis was performed by calculating the average red chromaticity level over 900 pixels of the cropped images. To examine the linearity of the red chromaticity level and the H_2O_2 concentration, the average and standard deviation of the red chromaticity level over 3 repeated experiments were plotted against the H_2O_2 concentration as shown in Fig. 6B.

A good linear relationship between the red chromaticity level

and the H_2O_2 concentration ranging from 10-80 μM with R^2 = 0.9895 was observed. The limit of detection (LOD) for determining H_2O_2 by the chromatic analysis was 6.19 μM. It should be strongly noted that by utilizing the colour information extracted from the digital images, the H_2O_2 concentration could be determined with comparable accuracy and precision to the results gained from spectroscopic information. Our developed approach demonstrates a method that incorporates inexpensive instrumentation, portability, unlimited sampling capability, rapidity, and straightforward quantitative chemical analysis. Our study also offers an alternative way to use the colour information extracted from a digital image captured by a small hand-held digital camera in order to construct the quantitative model to determine the analyte concentration, which is H_2O_2 in our case. Table 1 summarizes the detection limit (LOD) of our optimized procedure and of other comparable procedures based on utilizing silver nanoparticles combined with either spectroscopic technique or colorimetric approach. From Table 1, our protocol is comparable with the other works utilizing silver nanoparticles and UV-visible spectroscopy for H_2O_2 detection. In fact, the sensitivity of our sensors can be improved by decreasing the concentration of AgNPrs because the greater concentration of AgNPrs, the higher H_2O_2 concentration is required. However, a concentration (10 ppm) of AgNPrs was employed in order to prevent the color distortion of AgNPr solution under the light illumination. In addition, color intensity is appropriate for chromaticity analysis and naked eyed determination of H_2O_2.

Table 1. Comparison of other silver nanoparticles-based sensors for the determination of hydrogen peroxide (H_2O_2)

Material	Detection mechanism	Detection technique	Limit of detection (LOD)	Ref.
AgNS [a]	Decomposition of particles	UV-vis spectroscopy	1 μM	[25]
AgNS	Decomposition of particles	UV-vis spectroscopy	0.9 μM	[32]
AgNS	Aggregation of particles	UV-vis spectroscopy	10 nM	[42]
AgNPrs [b]	Decomposition of particles	UV-vis spectroscopy	0.2 μM	[14]
AgNS	Decomposition of particles	UV-vis spectroscopy	10 μM	[43]
AgNS	Decomposition of particles	UV-vis spectroscopy	1 μM	[44]
Red-AgNPrs	Decomposition of particles	UV-vis spectroscopy	1.57 μM	This work
Red-AgNPrs	Decomposition of particles	Chromaticity analysis	6.19 μM	This work

[a] AgNS = silver nanospheres; [b] AgNPrs = silver nanoprisms

We further investigated the sensitivity and performance of the developed chromatic analysis protocol for the quantitative determination of glucose utilizing AgNPrs. The shape transformation and corresponding solution colour of AgNPr were caused by the etching reaction of H_2O_2 generated from the enzymatic oxidation of glucose (D-glucose + O_2 + H_2O + Glucose oxidase enzyme → D-gluconic acid + H_2O_2.)[14] A series of glucose solutions with concentrations ranging from 20 to 100 μM were added to the homogenous AgNPr solution mixed with glucose oxidase enzyme and incubated for 60 minutes. As shown in Fig. 7, a distinct colour change of the AgNPr solution and a red chromaticity level shift can be observed as the concentration of glucose increased. A good linearity between the red chromaticity level and the glucose concentration ranging from 20–60 μM was observed. Therefore, glucose could also be determined at a micro-molar level using the chromatic analysis approach with an accuracy and precision comparable to reported nanoparticle-based techniques[14].

Furthermore, we also investigated the effects of glucose analogues species e.g. fructose, lactose, maltose and sucrose on the red chromaticity level of the AgNPrs. These species were separately added to the AgNPrs with glucose oxidase enzyme and the red chromaticity level of AgNPrs was measured under identical conditions (Fig. S7 in ESI†). It can be seen that the red chromaticity level dramatically dropped only in the glucose system, while the red level remains almost unchanged in the other systems. This suggests that this protocol gives very high selectivity for glucose determination.

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Conclusions

Starch-stabilized silver nanoprisms with red wine colour (Red-AgNPrs) were synthesized and employed for the determination of H_2O_2 utilizing colorimetric and chromatic approaches. Red-AgNPrs with a LSPR extinction maximum at 504 nm could be etched to smaller nanodisks in the presence of H_2O_2. The AgNPr solution colour consecutively transitioned from red to orange, and then to yellow, systematically corresponding to exposure to increasing H_2O_2 concentrations. H_2O_2 preferentially disintegrated AgNPrs at the deflected corner or edge, resulting in the rounding of AgNPrs to nanodisks with decreased lateral lengths. The decomposition profiles of AgNPrs by H_2O_2 were employed to establish a novel colorimetric method for H_2O_2 determination. A good linear relationship of the in-plane dipole LSPR wavelength shift and H_2O_2 concentration in the range of 10 to 80 μM with R^2 = 0.9910 was observed. The limit of detection (LOD) was determined to be 1.57 μM. In addition to the conventional quantitative analysis utilizing spectroscopic data, an alternative strategy based upon the combination of digital imaging and chromatic analysis of the colour change of AgNPrs for H_2O_2 quantification was also proposed. The accuracy and precision of the chromatic analysis approach utilizing the red chromaticity level as a quantitative index were comparable to the conventional spectroscopic approach in the detection range of 10-80 μM H_2O_2. The developed chromatic analysis approach can be further extended for the quantitative determination of glucose employing the oxidase enzyme mechanism. The developed chromatic analysis approach for H_2O_2 determination is rapid, simple,

inexpensive, and fast. In addition, the method possesses high sensitivity without using any spectrophotometer for detection. Therefore, we believe that the concept and the principal of the proposed colorimetric analysis are reliable and practical for demanding applications especially in the fields of biochemical analysis or clinical diagnosis.

Notes and references

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†Electronic Supplementary Information (ESI) available: Normalized UV-visible spectra of red-AgNPrs for three repetitions; The influences of (A) pH and (B) temperature on the AgNPrs with the insets of AgNPr solution color at various pH and temperatures; (A) Time-resolved LSPR spectra of AgNPrs after addition of 100 μM H_2O_2 from 0 to 15 min of incubation time. (B) Variations of absorbance and peak position of the in-plane dipole LSPR with incubation time. (C) Variations of absorbance and peak position of the out-of-plane quadrupole LSPR with incubation time; Extinction spectra of the original red-AgNPrs, red-AgNPrs interacting with hydrogen peroxide, and the solution after the conversion of silver ions to silver nanospheres by the addition of a reducing agent ($NaBH_4$); Extinction spectra of blue-AgNPrs when exposed to H_2O_2 at various concentrations ranging from 1 to 1,000 μM, with the corresponding inset photographs showing the colour of the colloidal AgNPr solution after incubation with H_2O_2 for 60 min; Normalized ATR FT-IR spectra of virgin starch, starch on AgNPrs, and starch on AgNPrs after incubation with H_2O_2 at various concentrations ranging from 1 to 1000 μM for 60 min. The infrared band assignment table is also included; Red chromaticity level of the AgNPrs with glucose oxidase enzyme after incubating with glucose and various potential sugar species. Inset photo shows the corresponding digital images of the AgNPrs solutions. See DOI: 10.1039/b000000x/

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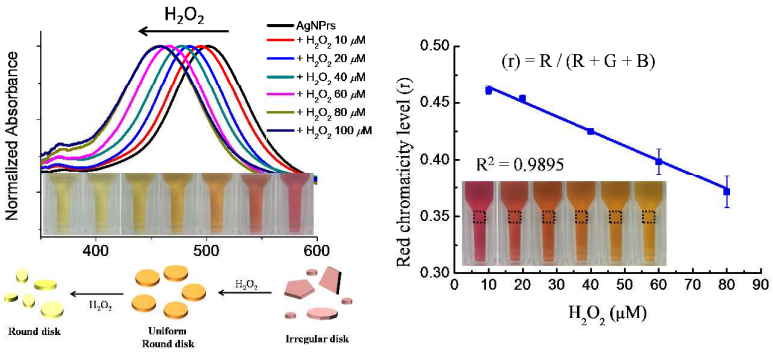
Graphical Abstract



Highly sensitive and accurate detection of hydrogen peroxide using starch-stabilized silver nanoprisms (AgNPrs) combined with image color analysis is proposed. The morphological changes of AgNPrs leaded to an appreciable colour change of AgNPr solution. H_2O_2 concentration at 1.57 μM can be recognized by naked-eye observation with good accuracy, stability and reproducibility.