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ORIGINAL PAPER

Gold nanoparticle-based thermal history indicator for monitoring low-temperature storage

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Abstract We describe a gold nanoparticle (AuNP)-based thermal history indicator (THI) for monitoring low-temperature storage. The THI was prepared from tetrachloroaurate using gelatin as a reducing reagent. Gelatin also acts as a stabilizer to control the growth of the AuNPs. The size and shape of the AuNPs were characterized by UV–vis spectrophotometry and transmission electron microscopy and are initially found to be spherical with an average particle size of ~19 nm. Initially, the color of the THIs is slightly pink, but after a 90-day storage in the freezer, as both the size and shape of the AuNPs change, the color of the THIs turns to red. After 90 days the absorbance peaks of THIs held at room temperature are red-shifted from 538 to 572 nm and possessed larger amplitude compared to those stored in the freezer. The color change is a function of both storage time and temperature. The observed increase in size is mainly due to storage temperature while the change in shape is mainly due to storage time. The THIs experiencing higher temperature treatments exhibit a more intense color change which is attributed to a localized surface plasmon resonance effect. Thus, the observed visual color changes can provide information regarding the thermal history the material has experienced. Accordingly, when used in conjunction with time-temperature sensitive products, the THI may serve as a proactive system for monitoring and controlling product quality and/or safety. For example, the THI is useful in safeguarding high-value biological products such as enzymes, antibodies, plasma, stem cells and other perishables that have to be stored at low temperatures.

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Introduction

Temperature at which biological materials and perishable foods are stored is critical to ensure their quality and/or safety. Biomaterials such as stem cells, plasma, enzymes, antibodies etc., need to be stored at low temperature for their safe-keeping. For example, for long-term storage, stem cells should be cryopreserved and stored at below −120 °C in liquid nitrogen or at −80 °C in mechanical freezers [1]; fresh frozen plasma can be stored safely for 3 months at −22 to −29 °C [2]; and most commercial enzymes and antibodies are generally stored in a freezer at −20 °C. The quality of these high-value biomaterials is also very sensitive to temperature fluctuations. For instance, if stem cells are stored overnight at room temperature, there is a significant reduction in cell recovery and viability [3].

Many foods and pharmaceuticals, which are widely used by the general public, also require low-temperature storage. In fact, storage temperature, along with storage duration, is the primary factor to ensure food safety and quality. The Food and Drug Administration (FDA) has defined a class of foods that requires time and temperature control for their safety as TCS (time/temperature control for safety) foods, because these foods are in a form capable of supporting rapid and progressive growth of infectious or toxigenic microorganisms (FDA Food Code 2013). A wide array of natural, processed, raw, and cooked foods of both animal and plant origin have been classified as TCS foods. Because of their prevalence in our daily diet, TCS foods pose serious threat to the safety of our food supply. Unfortunately, many foodborne illness outbreaks can only be identified after the health of the consumers are affected. This passive approach to food safety problem is often very

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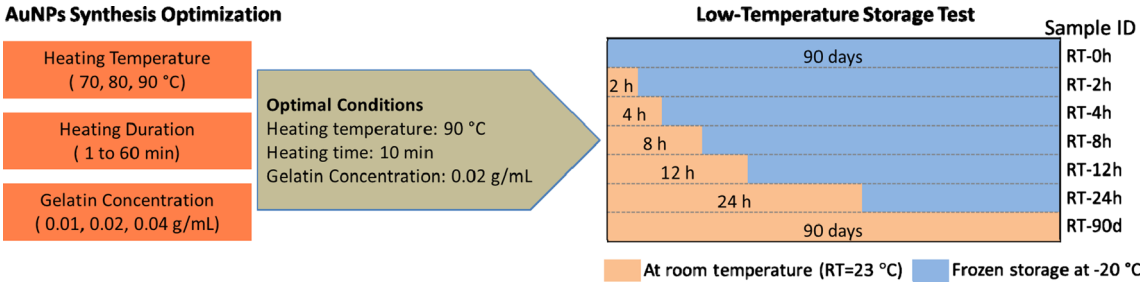

Fig. 1 The overall experimental design for gold nanoparticles (AuNPs) synthesis optimization and low-temperature storage tests conducted over 90 days with different durations at room temperature followed by frozen storage. The samples are designated as per Sample ID on the right

risky, expensive, and sometimes even fatal. Hence, it is vitally important to have a system to ensure and secure the safety of our food supply before they enter the food chain. Without such a proactive system to monitor food safety, the adverse effects of foodborne disease outbreaks, which cost at least $14 billion annually in terms of illnesses and loss of 61,000 quality-adjusted life in the United States [4], cannot be mitigated.

Generally, customers buy and use perishable foods and biomaterials based on the use-by-date printed on the product package. The listed product expiration date is an estimate based on several simple assumptions such as the product is being stored constantly at safe temperature until consumption. However, in reality many products experience temperature variations during handling, shipping, and storage. For example, accidental interruption in power, breakdown of transportation vehicles, employee error etc. can lead to unknown and uncontrolled temperature fluctuations. Thus, to better assess the quality/safety of foods, pharmaceuticals, and biomaterials, the consumers need information pertaining to time-temperature abuse the material has suffered rather than just the sell-by or use-by date.

To address this issue, thermal history indicators (THIs), which can help monitor the temperature- and time-dependent changes in material quality and/or safety are being developed. Use of radio frequency identification (RFID)-based electronic monitors can track product temperature [5]. However, these devices are expensive therefore not suitable for use with each product or package. Optical THIs, i.e., those based on color change either in the visible or outside of visible range, are considered less expensive and more user-friendly alternatives. Researchers have used enzyme-, dye-, and phase-separation-based methods to fabricate optical THIs [6–8]. However, THIs that exploit the unique and advantageous properties of nanomaterials are still being developed. Much research has been done on using nanomaterials as temperature sensors [9]. For example, cadmium telluride (CdTe) quantum dot can respond to a wide range of temperatures optically; however, the reaction is mostly reversible and hence it cannot record thermal history. Zhang et al. used a nanomaterials-based device that can record time and temperature changes [10]. However, their synthesis processes involve some toxic or hazardous chemicals, such as sodium borohydride and silver nitrate. Furthermore, in the published research little or no emphasis has been placed on temperature abuse occurring during low-temperature storage, which is critical for biological, pharmaceutical and food industry.

Herein we describe a facile method to prepare nanomaterial-based THI using gelatin. Gelatin is an inexpensive, non-toxic, biocompatible, and edible protein with high affinity to other proteins and gold nanoparticles (AuNPs) [11, 12]. This THI, compared to other types of THIs, could be used with each product/package, for simple and cost-effective monitoring of product's temperature history. Since low-temperature storage is very critical for many high-value biomaterials, pharmaceuticals, and foods, we focused on optimizing the THIs for different low-temperature/frozen storage regimes.

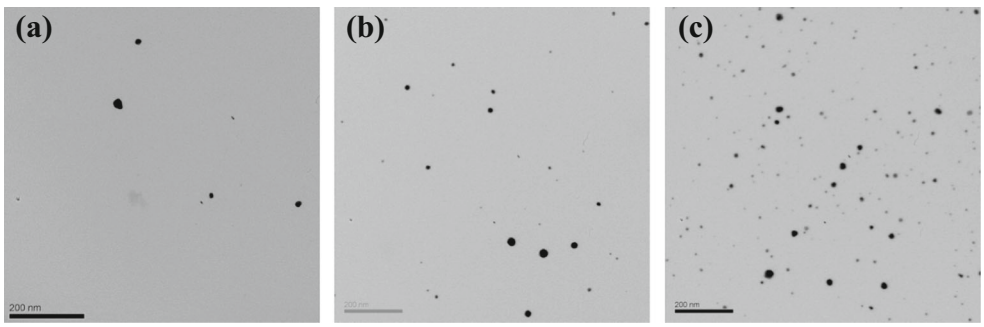

Fig. 2 TEM images of AuNPs during the synthesis of THIs (0.1 *v*/*v* 10 mM of $HAuCl_4$ within 0.02 g·mL^{-1} gelatin) after (**a**) 20, (**b**) 40, and (**c**) 60 min of heating at 90 °C

Table 1 Average size and size dispersion of AuNPs within different heating time of THIs

Heating time (min)	Particle size (nm, mean ± std. dev.; n=50)
20	19.5±4.2
40	16.3±6.8
60	13.5±7.0

Experimental

Reagents

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) and gelatin type A powder were purchased from Acros Organics. Both chemicals were used as received without any purification, and all aqueous solutions were prepared in deionized Millipore water (resistivity > 18 MΩ.cm).

Preparation and optimization of THIs

Gold precursor solution (10 mM) was prepared by dissolving $HAuCl_4$ in water. Gelatin solution was also prepared by stirring gelatin powder in water for 30 min at 90 °C for complete dissolution. In a typical procedure, 0.1 *v*/*v* gold precursor solution is mixed with the gelatin solution and heated in a water bath at 90 °C for 10 min under constant stirring, and 1 mL of this mixture is placed in 1.5-mL cuvettes, which constituted THIs. The effects of heating temperature (70, 80, and 90 °C for 60 min), heating time (1 to 60 min), and gelatin concentration (0.01, 0.02, 0.04 $g \cdot mL^{-1}$) were investigated.

Evaluation of THIs

The THIs prepared under optimal set of experimental conditions were evaluated for their performance subjecting them to different storage temperature regimes presented in Fig. 1. One set of THIs were exposed to room temperature (RT=23 °C) for 0, 2, 4, 8, 12 and 24 h before being stored in a freezer at −20 °C for a total of 90 days. These THIs were designated as RT-0 h to RT-24 h, with the number representing the duration the THIs were at room temperature prior to frozen storage. A second set of THIs was left at room temperature for 90 days, without ever being stored in the freezer; this was denoted as RT-90d. The color of all THIs were examined by monitoring their absorbance spectra using PerkinElmer, Lambda 25 UV/Vis spectrophotometer. The morphology of the nanoparticles was imaged using FEI Tecnai T12 transmission electron microscope (TEM) operating at 120 kV. Typically, one drop of sample was placed on a carbon-coated 400-mesh copper grid and excess solution was removed by wicking with filter paper. The grid was allowed to dry at RT before imaging. Particle size analysis was performed on at least 50 particles observed in the TEM images using Olympus Soft Imaging Viewer software and the particle size characteristics (mean diameter and standard deviation) were determined.

Results and discussions

Optimization of THI synthesis conditions

Heating temperature and heating time

Heating temperature and duration are important parameters during the preparation of THIs. As shown in Figure S1 (Electronic Supplementary Material, ESM), the synthesis of AuNPs commences sooner at higher heating temperature (90 °C), which is accompanied by the appearance of pinkish color [13]. Also, for the same heating duration, the color of THIs becomes more intense at higher heating temperature. The peak shift in localized surface plasmon resonance (LSPR) in the UV–vis spectra (Figure S2a, ESM) is consistent with the color change of the THIs. Therefore, 90 °C was chosen as the optimal temperature for the preparation of THIs as it takes less time.

Furthermore, at any given temperature, the pinkish color of THIs became more intense with time, which is more easily observed at 90 °C (Figure S1). The TEM images show that the average size of AuNPs decreased with increasing heating duration (Fig. 2). However, there are a large number of tiny particles, some of which grow larger with heating time. This

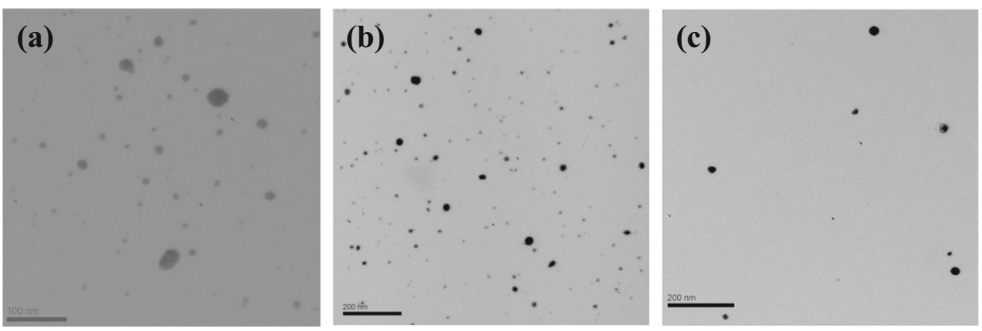

Fig. 3 TEM images of AuNPs during the synthesis of THIs (0.1 *v*/*v* 10 mM of $HAuCl_4$) with different gelatin concentrations ($g \cdot mL^{-1}$): (**a**) 0.01, (**b**) 0.02, and (**c**) 0.04 after 30 min of heating at 90 °C

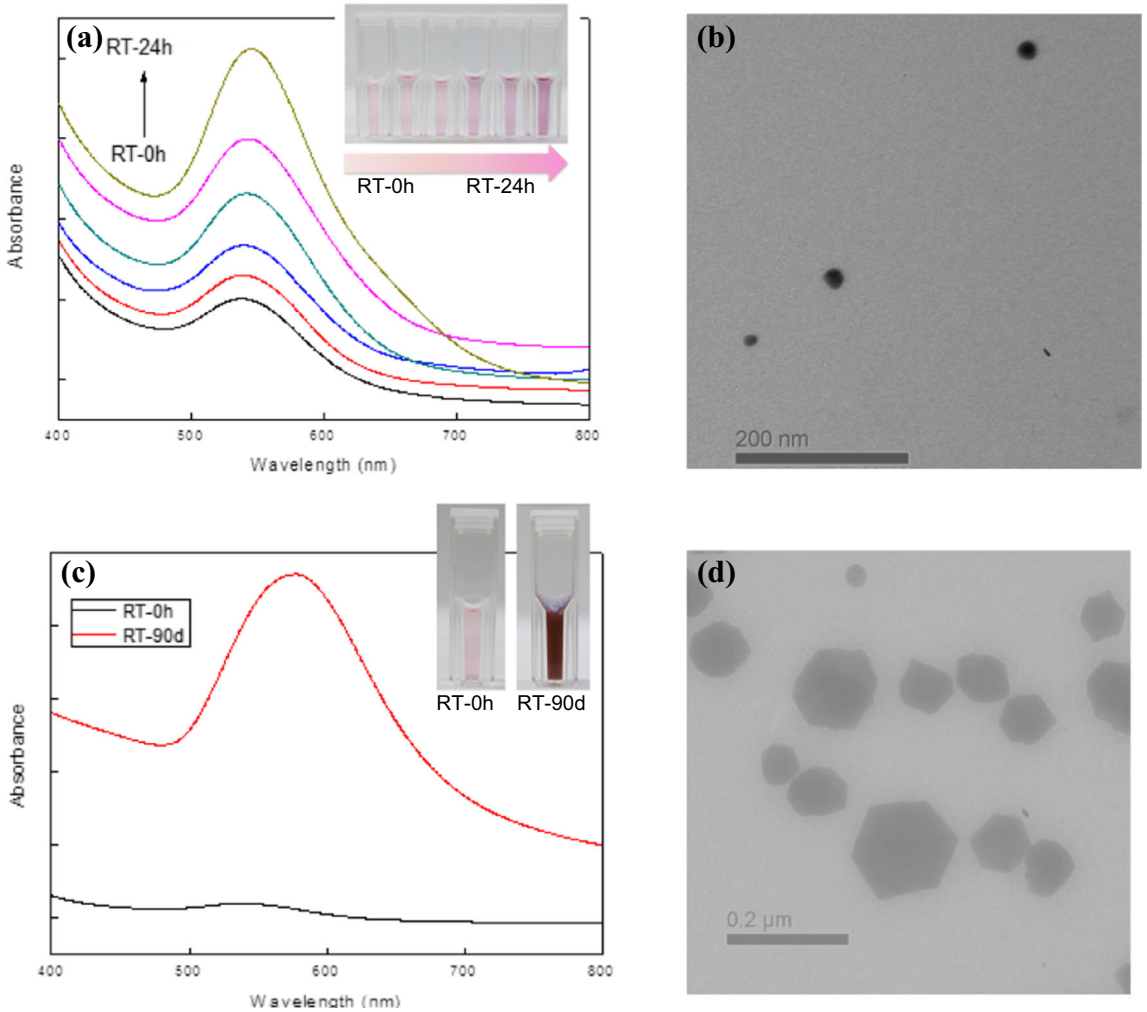

Fig. 4 **a** UV–vis spectra, and corresponding color (inset) of THIs after storage at room temperature for 0 to 24 h followed by in a freezer at −20 °C for 90 days. **b** TEM image of an as-synthesized THI showing fairly spherical and small AuNPs. **c** UV–vis spectra and corresponding color (inset) of the THIs stored for 90 days at room temperature (RT-90d) and in a freezer at −20 °C (RT-0 h). **d** TEM image of the RT-90d sample showing large and polygonal-shaped AuNPs

could explain why the color of THIs continues to grow more intense with increased heating time. As we had reported [14], during heating the reduction of gold precursor continues to progress, causing many small nuclei to form new particles or leading the existing small particles to attach to other larger particles. As a result, the size dispersion increases with heating

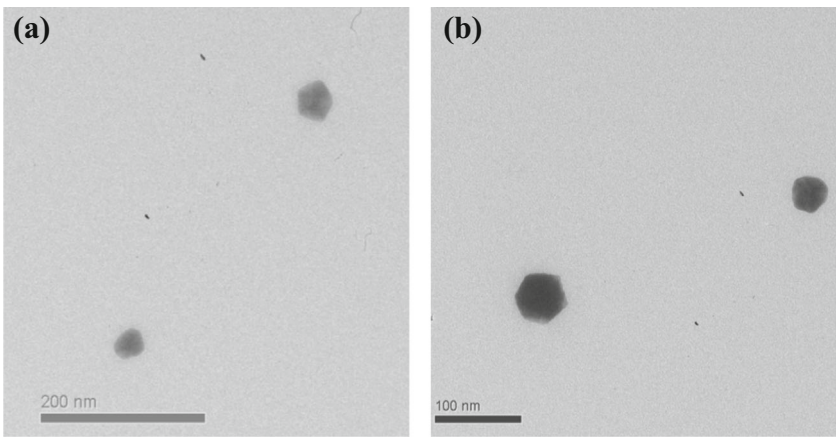

Fig. 5 TEM images of the gold nanoparticles in THIs (0.1 *v*/*v* 10 mM of $HAuCl_4$+0.02 g·mL^{-1} gelatin and heated at 90 °C for 10 min) after storage at room temperature for (**a**) 2 h and (**b**) 24 h followed by in a freezer at −20 °C for 90 days

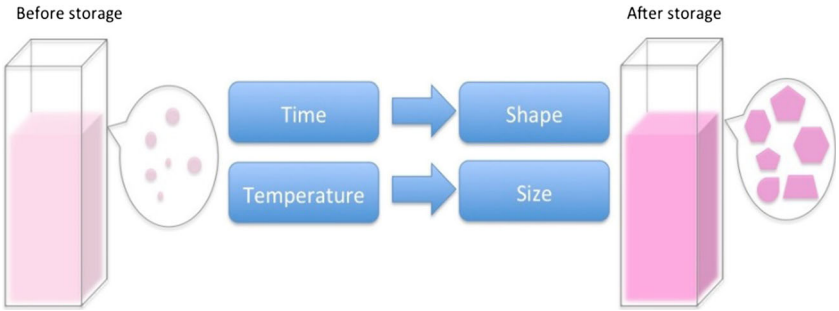

Fig. 6 Illustration of AuNPs size and shape evolution in THIs exposed to different storage times and temperatures. Generally, in our system, the size change is mainly due to storage temperature and the shape change due to storage time

time, from 4.2 nm (20 min of heating) to 7.0 nm (60 min of heating). The average size and standard deviation of AuNPs determined form the TEM images are listed in Table 1.

The UV–vis spectra show that the peak wavelength of the samples heated for 10 to 60 min are all ~537 ± 1 nm (Figure S2b, ESM); however, the amplitude of the spectra increased, which imply more nanoparticles are being synthesized [15]. Although some particles become larger, since the average size did not increase, the LSPR peak of the AuNPs did not show an obvious red-shift during this heating period. Moreover, typically a blue-shift in the LSPR peak is associated with either decomposition of large nanoparticles into smaller ones or change in the crystallinity of the nanoparticles [15]. Since the LSPR peaks did not show a blue-shift, we consider that the formation of many small particles during heating is not at the cost of few larger particles being decomposed. We continue to investigate the changes in crystallinity to more fully understand the reasons for these observed changes in the LSPR.

Gelatin concentration

Under the same heating regime, the THIs containing 0.01 g·mL^{-1} of gelatin produced the deepest purple color; whereas, the system with 0.04 g·mL^{-1} gelatin failed to develop the typical pinkish/purplish color (Figure S2c, ESM). We premise that the high viscosity of the THIs with high gelatin content hindered the growth of AuNPs. The TEM images (Fig. 3), which show larger particles in THIs containing 0.01 g·mL^{-1} of gelatin than in those containing 0.02 and 0.04 g·mL^{-1} of gelatin, corroborate this speculation. Although higher gelatin concentration could facilitate increased reduction of the gold precursor solution, the growth of AuNPs is more favored in systems with lower gelatin concentration. However, to better distinguish the color change by the bare eye and better observe the time- and temperature-dependent growth of AuNPs, we need to choose gelatin concentration that would not result in too fast or too slow a synthesis and growth of AuNPs. The THIs with 0.01 g·mL^{-1} gelatin produced too dark an initial color for further color development and visual observation; while the THIs with 0.04 g·mL^{-1} gelatin yielded too slow a rate of AuNPs synthesis. Hence, we chose 0.02 g·mL^{-1} as the optimal gelatin concentration for the preparation of THIs.

Evaluation of THIs

The UV–vis spectra of the samples (Fig. 4a) show a gradual shift in the LSPR of AuNPs – the longer the exposure of THIs at RT, the greater the red-shift. For example, comparing RT-0 h and RT-24 h, after 90 days of storage, their peak

Table 2 Comparison of different THIs reported in the literature

Method/ Mechanism	Matrix	Temperature Range[a]	Time Regime[a] and notes	Ref.
Enzyme-based	*Aspergillus oryzae* α –Amylase in polyacrylamide gel	7–65 °C	The enzyme activity may reduce with time	[6]
Dye-based	4,4′-Bis (2-benzoxazolyl) stilbene/ ethylene/norbornene copolymer	130–200 °C	Tunable between seconds and days	[7]
Phase Separation	Amide aqueous solution/ sodium chloride	Tunable from −10 to 60 °C	Once exposed to a certain temperature threshold, the phase separation reaction begins (It may only work at one specific temperature at a time)	[8]
Diffusion	lactic acid	4 to 45 °C	within 1 week	[18]
LSPR[b]	Ag/Au nanorods	10 to 35 °C	about 3 days	[10]
LSPR	Gelatin/AuNPs	− 20 °C to up to 90 °C	at least 90 days	This work

[a] Per experimental data reported

[b] Localized Surface Plasmon Resonance

wavelength has red-shifted from 538 to 546 nm. The corresponding colors of the THIs are also visibly distinguishable (Fig. 4a inset). These results suggest the potential for tracking temperature history based on system color and color intensity.

The just-synthesized THIs is light pink in color, perhaps due to the presence of AuNPs [13], which are fairly spherical with an average particle size of 18.9±3.4 nm (Fig. 4b). After 90 days of frozen storage, both the size and shape of AuNPs changed. Although some of the particles are irregularly shaped, many pentagonal and hexagonal particles were observed in the TEM images of different THIs. While we continue to investigate the mechanism for shape evolution, previous studies have suggested that time might be the key factor [16, 17].

The average size of AuNPs increased with the duration of exposure to RT, which perhaps corresponds to the change in the LSPR. Thus, we believe that duration of exposure of THIs at RT could be related to the particle size and hence the color intensity. This was confirmed by the UV–vis spectra and color (Fig. 4c) and the size of AuNPs (Fig. 4d) in RT-90d. The UV–vis spectrum of RT-90d has red-shifted to 572 nm with much larger amplitude compared to that of RT-0 h, whose LSPR peak is at 538 nm. This is an indication that more AuNPs have been synthesized [15].

The TEM images of RT-90d (Fig. 4d) show many hexagonal/ hexagonal-like AuNPs are seen; however, its average particle size is much larger than those in RT-0 h (Fig. 4b). Since the only difference between these two THIs is the temperature during storage, temperature is the key contributor to the size of AuNPs, which validates our previous suggestion, i.e., the longer the exposure of THIs to room temperature, the larger the size of the average AuNPs. Even 24 h of exposure to room temperature, compared to 2 h exposure, resulted in larger AuNPs (Fig. 5). The longer exposure to room temperature perhaps increases the reducing effect of gold precursor.

Thus, the color/UV–vis spectra of the THIs, is a function of both storage time and temperature (Fig. 6), which demonstrates the potential of THIs as a system for tracking thermal history of perishable foods, high-value biological and pharmaceutical products. Compared to several reported THIs (Table 2), our THI is simpler to fabricate and use over a wider range of temperatures and for longer storage periods, which is necessary for monitoring time-temperature abuse in products during low-temperature storage.

Conclusions

We used gelatin as a reducing agent and stabilizer to control the particle growth to prepare AuNPs-based THIs that are suitable for monitoring product quality and/or safety of food and biomaterials during low-temperature storage. The THI preparation parameters, heating temperature, heating time, and gelatin concentration, were optimized. We consider THI systems containing 0.1 *v*/*v* 10 mM of $HAuCl_4$ and 0.02 g·mL^{-1} of gelatin heated for 10 min at 90 °C to be the optimal for visually observing color changes during low-temperature (freezer to room temperature) storage. The THIs that were at room temperature for longer time exhibited deeper color along with more red-shifted LSPR peak of AuNPs. By comparing the TEM images and the UV–vis spectra of the AuNPs, our results demonstrate that the size and shape of the AuNPs are mainly the result of storage temperature and time, respectively. These THIs afford a proactive way for tracking time-temperature history of perishable foods and biomaterials by visual color change, and hence provide objective traceability information to determine quality and/or safety of products.

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