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Rapid Single-Step Detection of Hydrogen Peroxide Using Silver Nanoparticles

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ABSTRACT

Foods and beverages with nutritional value can have disastrous effects on health if adulterated. Present study reports application of silver nanoparticles colloids in testing hydrogen peroxide adulteration in milk samples as a model system. A good level of sensitivity was established at concentration as low as 0.01 M of hydrogen peroxide. Concentration higher than the permissible level i.e. 0.1 % (w/v) or 0.29 M can also be detected qualitatively and quantitatively. The method reported is very simple one-step, cost effective assay, as it requires no complex sample processing and is very rapid with visual detection.

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Introduction

Milk serves as a perfect medium for the rapid multiplication of dangerous microbes due to its high content of important elements, including proteins, lipids, carbs, minerals, and vitamins. Raw milk could get contaminated before or after mulching from cattle feed. This contamination could be natural or artificial leading to spoilage if not treated properly. However, there is a widespread practice of adulterating milk to reduce costs or extend the shelf life of dairy products. Hydrogen peroxide is one of the most common adulterants that has been found in milk. It has a long history of preserving stored milk with H_2O_2 before producing cheese. Hydrogen peroxide is routinely used for milk preservation, the permissible levels being 0.1% or 0.29 M. However, excessive H_2O_2 concentrations in milk can alter the chemical nature of milk as well as higher concentration can be harmful when consumed. Moreover, the quality of milk can further deteriorate with prolongation of treatment duration or increased temperature. In the dairy sector, hydrogen peroxide is frequently used as a disinfectant. As per FDA regulations, in order to produce dairy products, H_2O_2 must be removed during the pretreatment stage. At the same time, throughout the collection, transportation, and packaging of the milk, leftover hydrogen peroxide may enter into it [1-10].

Thus, this becomes a problem especially in tropical countries that lack advanced transportation and storage facilities. The rise in milk consumption has led to an increase in adulterations, primarily because local manufacturing and packaging processes find it challenging to implement detection techniques. The official procedures have limited utility in routine control since they are costly, time-consuming, and prone to producing false results. Various techniques for measuring hydrogen peroxide in milk have been developed through the application of colorimetric and electrochemical approaches. The development of analytical techniques that are quick, affordable, and precise is crucial for the detection or measurement of adulterants in milk. One important aspect of green chemistry research is to seek for novel analytical techniques that can minimize or replace compounds that are hazardous to the environment and human health. For the purpose of identifying H_2O_2 in adulterated milk, a highly sensitive nonenzymatic electrochemical sensor has been designed. As electrocatalysts, the bimetallic gold-platinum nanoparticles (AuPt NPs) are utilized to specifically improve the electrochemical signal of H_2O_2. In both ultrahigh temperature processing (UTH) and raw milk samples, the screen printed carbon electrode (SPCE) modified with the appropriate Au:Pt molar ratio of AuPt alloy nanoparticles (NPs) shows good electrocatalytic performance in electro-reduction of H_2O_2 ions as reported by Sangkaew et al . The development of rapid, low-cost, and accurate analytical procedures is essential for the identification or quantification of adulterants in milk. The hydrogen peroxide content of milk can also be determined using electrochemical techniques [11-16].

It was also explained how to employ spectrophotometric, luminescent, and colorimetric techniques. The primary drawback of directly applying all of these methods, is the inability to simultaneously determine a large number of indicators in a single analysis.

Additionally, there have been recent reports in India, that hydrogen peroxide is being used as adulterant to make the milk look thick. Such activities must be critically monitored to ensure safe distribution for the public to consume. There are some conditions under which hydrogen peroxide can be permitted for the preservation of milk. In developing countries where the production and collection of milk is not sufficiently organized as well as the preservation facility is inadequate, hydrogen peroxide can be added in safe monitorization. The majority of tropical nations produce milk in modest quantities on scattered farms, which must be collected in bulk and transported over extensive distances to plants for pasteurization and refrigeration. In tropical and less developed nations, where roads and transportation infrastructure are in poor condition, milk may not reach the consumption area quickly enough to maintain its shelf life. Tropical nations experience high seasonal temperatures in the atmosphere, which promotes the quick growth of bacteria and the rapid deterioration of milk [2,17-21].

The remarkable characteristics of metal nanoparticles (NPs) over bulk materials can be attributed to their enormous surface-to-volume ratios and degenerate energy densities. George and Chowdhury have reported successful incorporation of Au, Ag nanoparticles within MIL-53 Fe which showed excellent dispersive capability and morphological stability when embedded individually with Au and Ag for colorimetric detection of H_2O_2 in milk sample. A number of factors, including the kind of metal precursor, its concentration, the type of solvent, and the reductant utilized, influence the type of metal nanoparticles. Silver nanoparticles (AgNps), are widely used in the biological, chemical, and environmental areas due to their intense surface plasmon resonance (SPR) bands in the wavelength range of 300-900 nm. The SPR absorbance of AgNps highly sensitive to their shape, size and distance, based on which various sensors can be fabricated. The exceptional plasmonic characteristics of anisotropic silver nanoparticles (AgNPs) in visible and near-infrared (NIR) range have led to substantial research on structures like triangular prisms and plate-like nanostructures. Hence, this work focuses on development of a method by using silver nanoparticle colloids to detect hydrogen peroxide contamination in milk samples [22-28].

Experimental

Materials and Methods

Silver Nitrate ($AgNO_3$, 99.9% pure) was obtained from Sisco Research Lab Pvt. Ltd. Mumbai, India. Sodium hydroxide (NaOH), soluble starch and d- (+) glucose were obtained from HiMedia Laboratories, Mumbai, India. Hydrogen peroxide (6% (w/v) was obtained from Nice Chemicals Pvt. Ltd., Chennai, India. All chemicals were used without any further purification or treatment. Double distilled water was used for all the experiments

Synthesis of aqueous dispersion of starch stabilized silver nanoparticles (Ag-NPs).

The aqueous dispersion of silver nanoparticles was synthesized by the chemical reduction of $AgNO_3$ with d-glucose as a reducing agent, starch as a capping agent and sodium hydroxide as a base reaction catalyst. A scale up synthesis was done based on similar procedure adopted from Vasileva et al. and Sarma and Chattopadhyay, with a few variations to obtain higher concentration of nanoparticles. Figure 1 represents the schematic illustration of synthesis procedure. The synthesis procedure was carried out using a magnetic stirrer as well as sonicating bath under the operating frequency around 40 kHz and 100 W output power in separate batches. The comparison of profile of quantities of chemicals is tabulated in Table 1. All the experiments were done in triplicates and readings were recorded in replicates [29,30].

Table 1: Comparison of Concentrations of Chemicals Used in Normal and Scale Up Synthesis

Chemicals Used	Normal Synthesis (magnetic stirrer)	Scale up Synthesis (sonication bath)
Silver Nitrate	0.001 M	0.03 M
Soluble Starch	0.2% w/v	1.0% w/v
d-(+) glucose	0.1 M	1.5 M
Sodium Hydroxide	0.1 M	0.2 M

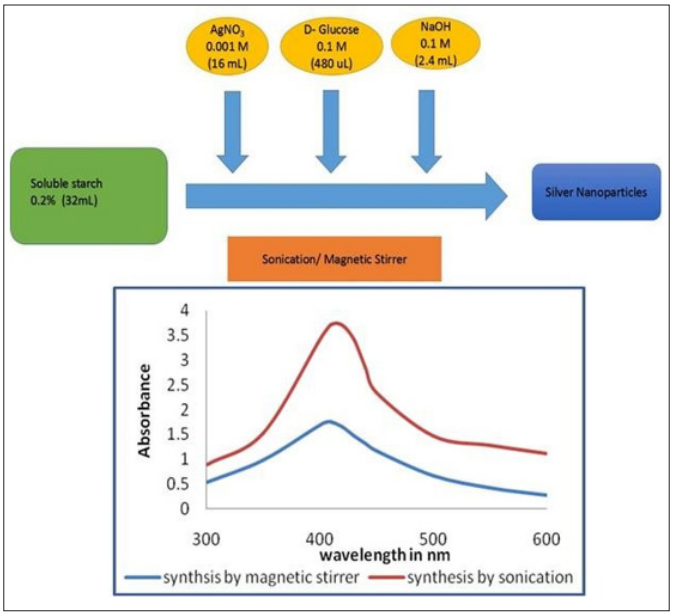

Figure 1: Schematic diagram of green synthesis procedure of silver nanoparticles and variation in the intensity of yellow colour of silver nanoparticles synthesized recorded by UV-Vis absorption spectra of silver nanoparticles synthesized using conventional magnetic stirrer and sonication

Characterization

UV-Vis Spectroscopy

The optical absorption spectra of the synthesized silver nanoparticles were recorded in Ultrospec 1100 pro UV-Vis spectrophotometer (Amersham Biosciences). The scanning range for the samples was 300-600 nm.

Scanning Electron Microscopy

Scanning Electron Microscopic (SEM) analysis was done using FEI Quanta FEG 200High Resolution Scanning Electron Microscope. Thin films of the sample were prepared on a copper grid following standard protocol.

Calibration curve to calculate the concentration of AgNPs in the synthesized dispersion

A mixture of water and Tween-80 was used to disperse commercially available silver nanoparticles (AgNPs) at varied known concentrations ranging from 1.5 mg mL^{-1} to 0.0625 mg mL^{-1}. The optical absorption spectra of AgNPs were recorded using UV-Vis spectrophotometer within scan range of 300 to 600 nm. The absorption maximum was observed at 410 nm, which was used for rest of the study.

Results and Discussion

Synthesis of aqueous dispersion of starch stabilized silver nanoparticles (AgNPs)

The change in colour obtained after the addition of sodium hydroxide was light yellow within 20-25 mins confirms the formation of silver nanoparticles in case of conventional magnetic stirrer. However, in case of sonication-mediated synthesis, as shown in Figure 1, the colloid produced was yellowish brown in color, and it was noted that the synthesis process took just 10 to 15 minutes. The above observation is due to the fact that, in comparison to traditional magnetic stirring, sonication offers superior homogenization of the reaction mixture and uniform chemical concentration throughout the solution. The concentration of AgNPs for synthesis mediated by magnetic stirrer was found to be 570 µg mL^{-1}, whereas the concentration for synthesis mediated by sonication was found to be 1.8 mg mL^{-1} (Table 2), as per the standard curve equation. Thus, more silver ions were reduced by glucose and the reaction was accelerated with the dropwise addition of sodium hydroxide during the scale-up synthesis; the colourless reaction liquid turned dark brown (Figure 2). This suggests a higher silver nanoparticle concentration and was further confirmed by UV-Vis absorption spectroscopy and SEM analysis (Figure 2). Stability of particles was also analysed spectroscopically (Figure 3). Maryan and Gorji reported synthesis of nano silver by reducing $AgNO_3$ with glucose and/ or cellulose, using the chemical reduction process. They too, used sodium hydroxide and reported size reduction of particles as was found in current study. Reducing agent was glucose and/ or cellulose in their case, whereas in the current study glucose was employed as reducing and starch was employed as the stabilizing agent. In both instances, the production of nano silver has been accomplished successfully. We have additionally reported the yield of particles. have also reported green synthesis of silver particles using four different reductants [31,32].

Table 2: Concentration of Silver Nanoparticles in the Synthesized Colloids

Silver nanoparticles colloid	Concentration of silver nanoparticles
Synthesized using magnetic stirrer	570 ±15 µg mL^{-1}
Synthesized using sonication	1.8 ± 0.1 mg mL^{-1}

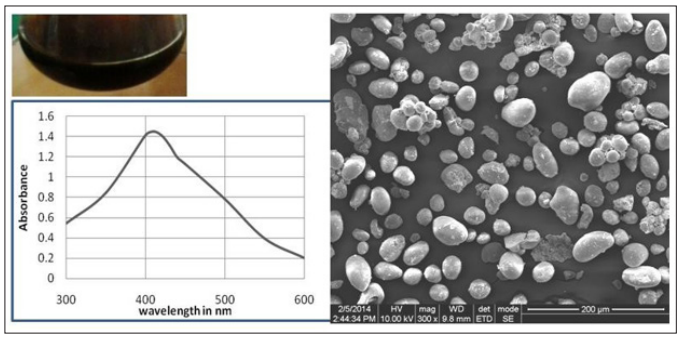

Figure 2: Scale up Synthesis. Dark brown colour of the silver nanoparticles colloid recorded as UV-Vis absorption spectrum after 1/16th dilution. SEM image of the same (right panel)

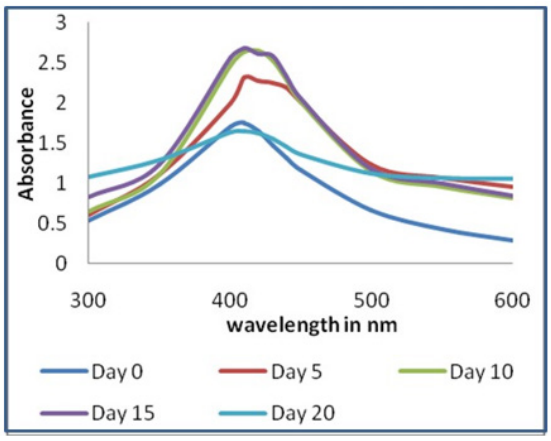

Figure 3: The comparison of change in stability after 20 days. UV-Vis absorption of silver nanoparticles synthesized taken at an interval of every 5 days

Calibration curve to calculate the concentration of Ag-NPs in the synthesized dispersion

A graph was plotted with optical density values at 410 nm from the stocks of different known concentrations (varying from 1.5 mg mL^{-1} to 0.0625 mg mL^{-1}) of AgNPs at y-axis and known concentration of AgNPs on x-axis to obtain the calibration curve. The standard equation of the curve was calculated as follows:

$$y = 0.663x + 0.0824$$

The concentration of colloidal silver nanoparticles was calculated by using the equation mentioned above and was found to be 17.1 mg mL^{-1}.

Evaluation of the optical characteristics of starch-stabilized AgNP colloid as an improvised peroxide sensor in milk

The experimental procedure with milk was done in two phases. In the first phase, milk was cleared of casein by addition of glacial acetic acid and then it was centrifuged at 11000 rpm and precipitate was removed via filtration leaving clear milk. In the second phase of the experiment, milk was diluted instead of clearing the casein. Figure 4 shows the schematic illustration of the procedure adopted. For the former 1 mL of 0.01mol/L of hydrogen peroxide was mixed with the clear milk which in turn was introduced into test tubes containing varying volume (250 µL-1000 µL) of synthesized silver nanoparticles colloids The change in absorbance was recorded by UV-Vis spectra. The graph for silver nanoparticles and milk with hydrogen peroxide was plotted as shown in Figure 5I. Quantification of the nanoparticles was done by using the standard equation mentioned above and, it was found out that 43 µg to 44 µg of silver nanoparticles were degraded in both (clear and diluted) samples, respectively (Table 3).

Table 3: Amount Of Silver Nanoparticles Degraded By 0.01m Hydrogen Peroxide

Sample (Volume of silver nanoparticles colloid used)	Amount of silver nanoparticles degraded by 0.01M hydrogen peroxide
For 250 µL [A]	43.5 ± 0.2 µg
For 500 µL [B]	44.0 ± 0.1 µg

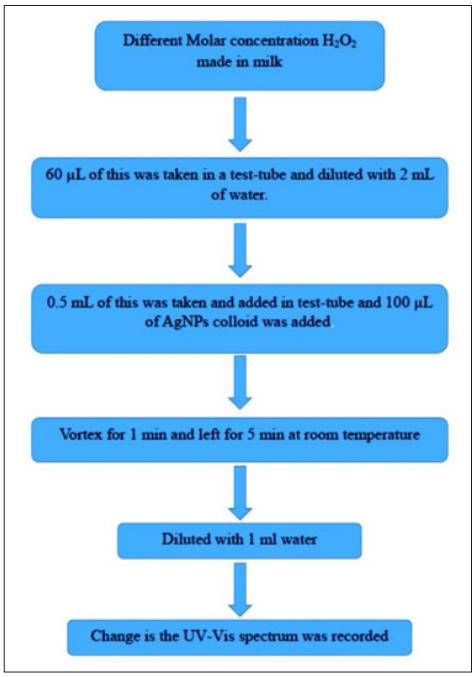

Figure 4: Schematic diagram for the second phase experiment

A qualitative or visual confirmation of hydrogen peroxide detection was seen in the second experiment with diluted milk samples. The results are represented in Figure 5II. With increase in the concentration of hydrogen peroxide the milk sample turned transparent. In addition, it was possible to calculate the amount of silver nanoparticles degraded in manner similar to the one used for clear milk samples indicated above. Here, for the milk sample treated with 0.01 M hydrogen peroxide the amount of particle degraded was found to be 38 µg. Figure 5II shows that fading of milk sample from white to colorless, in presence of AgNPs, is concentration dependent and the transparency of solution increases with increasing concentration of H_2O_2.

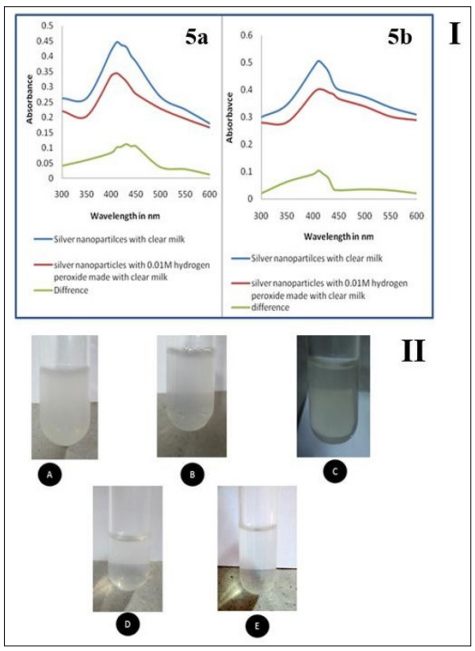

Figure 5: [Upper Panel (I)] Graph plotted for the volume of 250 µL and 500 µL of silver nanoparticles colloids treated with 0.01 M hydrogen peroxide made with clear milk. [A] Graph for 250 µL [B] Graph for 500 µL. [Lower Panel (II)] Visible changes noted in the diluted milk sample containing different molar concentrations of hydrogen peroxide, after being subjected to silver nanoparticles colloid. A) Milk sample without hydrogen peroxide. B) Milk sample with 0.01M hydrogen peroxide. C) Milk sample with 0.05 M hydrogen peroxide. D) Milk sample with 0.1 M hydrogen peroxide E) Milk sample with 1.0 M hydrogen peroxide.

Therefore, starch-protected Ag-NPs are degraded by the catalytic breakdown of H_2O_2, which results in the oxidation of silver to Ag^+ ions. Hence, from both the experiments, we conclude that even 0.01 M of hydrogen peroxide (which is lower than the safe or admissible level of hydrogen peroxide) can qualitatively and quantitatively be determined using this method. The advantage of this method is that the time required for the analysis (qualitative and quantitative measurement) of hydrogen peroxide in milk is minimal, and there are no complex sample processing steps. Have reported the amount of hydrogen peroxide present in different milk sample at different temperature and pH over a period of time. Wang et al. developed a sensor for detecting hydrogen peroxide using mediator free DNA and horseradish peroxidase immobilized on silver nanoparticles. Have fabricated silver nanoparticle into the microsphere of TiN via one pot solvothermal reaction and subsequent nitridation for electrochemical detection of hydrogen peroxide. Developed an optical test trip with silver nanoparticle for the quantitative detection of hydrogen peroxide in aqueous solution. By an in-situ approach, they have synthesized silver nanoparticles by reducing $AgNO_3$ in Nafion-117 membrane which is previously absorbed with ascorbate ion. The computed detection limit was reported to be 2.6×10^{-8} mol L^{-1}. As per their method, their test strip was able to effectively estimate the concentration of H_2O_2 in milk. As compared to the reports published and discussed here, the method reported in the current study adopts a very simple particle synthesizing approach, which is scalable yet cost effective. It was also possible to estimate yield of the particles. In addition to this, the application scheme of particles for quantifying H_2O_2 is extremely simple and therefore, can be used to determine hydrogen peroxide at point of sample collection. No sophisticated instrument or skill is required for the proposed analysis. Although in this study milk was used as the model system, but any liquid containing H_2O_2 can be detected using the same scheme [20,33-37].

Conclusion

In summary, present work proposes an easy and cost-effective technique for the detection of H_2O_2 in milk. This can be extended to other H_2O_2 containing liquid samples as well. Method adopted has significant advantages. There is no complicated sample processing and the time taken for qualitative and quantitative analysis of hydrogen peroxide in milk is short. Silver nanoparticle colloid could be used as such without need of conversion of colloid to powdered nanoparticles. The developed assay is highly sensitive. This work could serve as a base for future development of a more robust method where sensitivity, specificity and limit of detection are identified.

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