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DETECTION OF HYDROGEN PEROXIDE AS A POTENTIAL ROS WITH GOLD NANOPARTICLES AND COPPER (II) CHLORIDE CATALYST

Fuhad Hasan Fahim[1], Sumit Ghosh Kabbo[2], Abdullah Al Masud[3] & Nahid Sanzida*

[1]Department of Civil Engineering, Kansas State University, Manhattan, KS-66502

[2]Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX 77204-4004

[3]Department of Chemical Engineering, Bangladesh University of Engineering and Technology, Dhaka 1000

*Department of Chemical Engineering, Bangladesh University of Engineering and Technology, Dhaka 1000

Abstract

Reactive oxygen species (ROS) play an important role in various biological processes including cellular signalling, oxidative stress, and disease development and thus accurate detection and quantification of ROS species is crucial for understanding their impact on biological systems and developing effective therapeutic strategies. H_2O_2 is one of the major ROS species. The objective of this study was to develop a cost effective, highly sensitive and selective H_2O_2 detection technique utilizing gold nanoparticles with cupric dichloride as a catalyst. Gold nanoparticles were synthesized by classical Turkevich method and UV-Vis spectrophotometer recorded the wavelength and absorption of surface plasmon resonance (SPR) peak. The structure and surface morphology of synthesized GNPs was obtained from SEM analysis and compared with literature values. Different amount of catalyst ($CuCl_2$) was added to the reaction media and the shift in the absorption peak was recorded. This shift in the absorption peak was plotted against the concentration of hydrogen peroxide and it was observed that with increasing concentration of peroxide solution the shift in the peak also increases. Among the three different alternative amounts of $CuCl_2$ (100µL, 200 µL and 300 µL), 100 µL catalyst in the reaction media was chosen as the best possible alternative. A calibration curve was generated by plotting the absorbance data against respective concentrations of H_2O_2 which successfully validated the goal of our work.

Keywords: ROS, H_2O_2, Turkevich method, Surface plasmon Resonance, Calibration curve.

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1. Introduction

Reactive oxygen species are highly reactive, oxygen containing molecules that are the result of an incomplete reduction of molecular oxygen in the cell [3] [7]. Examples of ROS include hydrogen peroxide (H_2O_2), the superoxide (O_2• -), hydroxyl (HO•), alkoxyl radicals (RO•) etc [5] [8]. Reactive oxygen species have various functions in human as well as animal body such as acting as signalling molecules, creating oxidative damage which works as a biomarker of aging and maintaining 'Redox Homeostasis' which is done by keeping their concentration below a certain level [2] [4].

Due to well-known localized surface plasmon resonance (LSPR) characteristics, metallic nanoparticles possess unique optoelectrical properties. Nanoparticles of the alkali and noble metals i.e. Cu, Ag and Au have a broad absorption band in the visible zone of the electromagnetic spectrum. Due to their advanced optical properties, metal nanoparticles find applications in many research areas. The Turkevich method is one of the most commonly used methods for synthesis of spherical AuNPs in the size range of 10 nm-20 nm. In this method to synthesize AuNPs, $HAuCl_4$ aqueous solution is boiled, and trisodium citrate dihydrate solution is added under stirring, which reduces Au^{+3} ions to Au^0. The resulting colloidal suspension of gold is wine-red with a size of around 20 nm, and citrate ions act as stabilizing agents to prevent aggregation [1].

The morphological features of nanoparticles always attain great interest since morphology always influences most of the properties of the nanoparticles. There are different characterization techniques for morphological studies, but microscopic techniques

*Corresponding Author: *nahidsanzida@che.buet.ac.bd* such as polarized optical microscopy (POM), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) are the most important of these. Of them SEM technique is based on electron scanning principle, and it provides all available information about the NPs at nanoscale level. This technique is used to study not only the morphology of their nanomaterials, but also the dispersion of NPs in the bulk or matrix [6] [10].

Nanotechnology plays an important role in current sensor technology. Colloidal plasmonic nanoparticles (NPs), mainly gold and silver NPs (AuNPs and AgNPs), exhibit highly intensive color because of plasmon absorption bands located in the visible region (Saha et al., 2012). Due to this, they are extensively used in visual detection owing to its optical property known as Surface Plasmon Resonance (SPR). Colorimetric-based assays have been developed by exploiting the color changes associated with the aggregation of metal-noble NPs [9].

2. Methodology

2.1 Development of Colorimetric Assay

In this study, we aimed to develop a cost-effective, highly sensitive and selective H_2O_2 detection technique by utilizing GNPs with Copper (II) Chloride as a catalyst. GNPs were synthesized using the classical Turkevich method, and their surface morphology was observed through SEM analysis. The UV-Vis spectrophotometer was employed to record the wavelength and absorption peak, a characteristic feature of GNPs. By incorporating different amounts of $CuCl_2$ into the reaction media, we investigated the shift in the absorption peak. This shift was then correlated with the concentration of H_2O_2, to establish a calibration curve. We compared three alternative amounts of $CuCl_2$ (100 µL, 200 µL, 300 µL) and identified 100 µL as the optimal catalyst amount based on the observed shift in the absorption peak. To validate the effectiveness of our H_2O_2 detection technique, we evaluated the absorbance data of H_2O_2 solutions at two concentrations: 7 mM, which is within the calibration curve range and 20mM, which is outside of the calibration curve range. The successful fit of the absorbance data to the calibration curve confirmed the accuracy and reliability of our developed method.

2.2 Preparation of 4mM $CuCl_2$

0.276 gm of copper (II) chloride (MW: 134.35 g/mol) was weighed carefully and dissolved in 40 mL of deionized water.

2.3 Preparation of H_2O_2 solution

Initially the actual concentration of supplied solution was measured by titrating with $KMnO_4$ solution. And later the solution was diluted to form peroxide solution of different concentrations.

2.4 Transfer and Absorbance

2mL of GNP solution was transferred in 3 glass vials through a pipette and 100µL,200µL,300µL of prepared $CuCl_2$ solution was added to the solution with the help of a micropipette. The absorbance of the solutions was recorded.

2.5 Hydrogen Peroxide Addition and Absorbance

2mL of H_2O_2 solution was introduced to the mixture and absorbance data for 2 hours was recorded within an interval of 1 hour. During this timeframe the color change of the reaction media was observed.

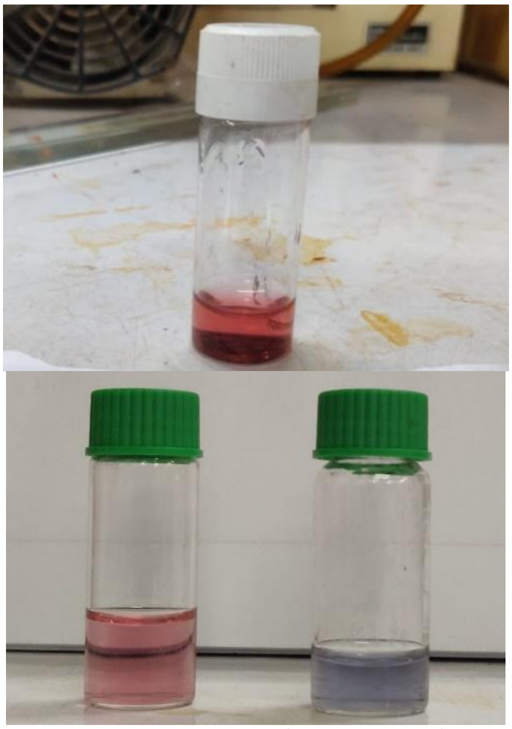

Fig. 1: (a) Pure GNP solution. (b) After the addition of $CuCl_2$ and H_2O_2 respectively

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From Figure 1(a), we see that the color of pure GNP solution is wine red. As $CuCl_2$ is added, the solution turns light which indicates the dilution of the solution. After the addition of H_2O_2, the red color completely disappears which symbolizes the completion of the reaction between GNP and H_2O_2.

3. Results and Discussion

3.1 Generation of the Calibration Curve

Absorbance peak was observed using UV-visible spectrophotometer for different H_2O_2 concentration added to GNP and $CuCl_2$ mixture. The absorbance data was plotted against the H_2O_2 concentration and a calibration curve was generated.

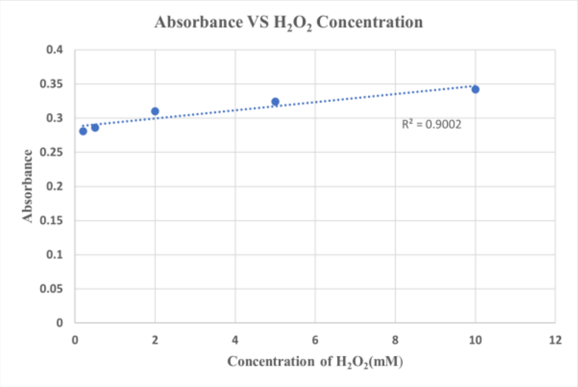

Fig 2: Change in absorbance VS H_2O_2

From the graph it is found that the change in absorbance of the nanoparticle solution increases with the concentration of hydrogen peroxide(analyte). As H_2O_2 is added to the solution, it interacts with the gold surface and as a result there is a change in the surface charge and structure of nanoparticles. This change in surface charge results in a shift of the surface plasmon resonance (SPR) peak of the nanoparticles which is measured by UV-Vis spectroscopy. And higher concentration of H_2O_2 means more binding of nanoparticles with the analyte, a greater shift in the peak.

3.2 Effect of catalyst

Cupric chloride acts as a catalyst in the nanoparticle solution and enhances the catalytic activity towards H_2O_2 detection because they can promote the formation of much smaller and uniform nanoparticles with higher surface area. They can selectively adsorb onto the surface of GNPs and improve the selectivity towards H_2O_2 detection. But from the figure it is seen that increasing the amount of catalyst decreases the specificity of this detection method because the addition of too much catalyst may had resulted in the aggregation of nanoparticles. And due to aggregation of nanoparticles, the number of active sites were reduced thus decreasing the sensitivity towards H_2O_2 detection. Moreover, from the graph it is being seen that addition of 200 and 300 µL of cupric chloride produces two almost overlapping plots which also signifies the addition of excess catalyst isn’t producing any significant change to the overall results.

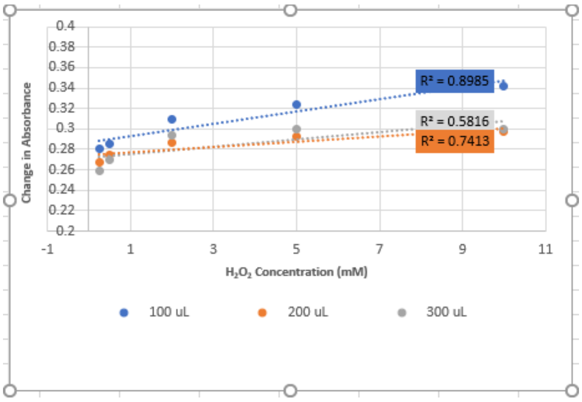

Fig 3: Effect of Catalyst (Cu_2Cl_2) on Volume and Concentration of H_2O_2

3.3 Validation of Calibration Curve

SSE = (0.00006241+ 0.00001547) = 0.00007788
Additionally, from Table 1, it is evident that using 2 values of peroxide concentration 7mM and 20 mM, one inside the range of calibration curve and other outside the range of calibration curve the value of SSE (Sum of Squared Errors) is very low. It implies that the differences between the predicted and actual values are small. In other words, the model's ability to explain the variance in the dependent variable is relatively high.

Concent-ration of H_2O_2 (mM)	Actual value of Absorba-nce Shift (y)	Experiment-al value of absorbance shift ($\hat{y}$)	$(y - \hat{y})^2$
7	0.3294	0.3215	0.00006241
20	0.4074	0.4113	0.00001547

4. Conclusions

This experiment has successfully demonstrated the utilization of gold nanoparticles (AuNPs) in the detection of hydrogen peroxide (H_2O_2), with cupric chloride ($CuCl_2$) as a catalyst. The unique properties of AuNPs, such as their stability, large surface area-to volume ratio, and tuneable surface plasmon resonance, make them highly suitable for H_2O_2 detection. The addition of cupric chloride as a catalyst further promoting the reaction between AuNPs and H_2O_2, leading to amplified signals and improved detection limits has also been discussed. Despite all these, some limitations still exist in the study. The experiment was not performed on any biological sample so, mitigating the impact of interfering species remains a challenge that requires further research and optimization. Additionally, translating the system into portable devices or integrating it into existing analytical platforms may pose engineering and logistical challenges that also need to be addressed. Overall, the system is cost-effective and relatively simple to implement, making it feasible for widespread use.

5. References

[1] Alex, S., & Tiwari, A. (2015). Functionalized Gold Nanoparticles: Synthesis, Properties and Applications—A Review. *Journal of Nanoscience and Nanotechnology*, *15*(3), 1869–1894. https://doi.org/10.1166/jnn.2015.9718

[2] Dröge, W. (2002). Free Radicals in the Physiological Control of Cell Function. *Physiological Reviews*, *82*(1), 47–95. https://doi.org/10.1152/physrev.00018.2001

[3] Nosaka, Y., & Nosaka, A. Y. (2017). Generation and Detection of Reactive Oxygen Species in Photocatalysis. *Chemical Reviews*, *117*(17), 11302–11336. https://doi.org/10.1021/acs.chemrev.7b00161

[4] Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science : IJBS*, *4*(2), 89.

[5] Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian Journal of Clinical Biochemistry*, *30*(1), 11–26. https://doi.org/10.1007/s12291-014-0446-0

[6] Saeed, K., & Khan, I. (2014). Preparation and properties of single-walled carbon nanotubes/poly(butylene terephthalate) nanocomposites. *Iranian Polymer Journal*, *23*(1), 53–58. https://doi.org/10.1007/s13726-013-0199-2

[7] Santos, A. L., Sinha, S., & Lindner, A. B. (2018). The Good, the Bad, and the Ugly of ROS: New Insights on Aging and Aging-Related Diseases from Eukaryotic and Prokaryotic Model Organisms. *Oxidative Medicine and Cellular Longevity*, *2018*, 1–23. https://doi.org/10.1155/2018/1941285

[8] Winterbourn, C. C. (2008). Reconciling the chemistry and biology of reactive oxygen species. *Nature Chemical Biology*, *4*(5), 278–286. https://doi.org/10.1038/nchembio.85

[9] Sabela, M., Balme, S., Bechelany, M., Janot, J.-M., & Bisetty, K. (2017). A Review of Gold and Silver Nanoparticle-Based Colorimetric Sensing Assays. *Advanced Engineering Materials*, *19*(12), 1700270. https://doi.org/10.1002/adem.201700270

[10] Saeed, K., & Khan, I. (2016). Preparation and characterization of single-walled carbon nanotube/nylon 6, 6 nanocomposites. *Instrumentation Science & Technology*, *44*(4), 435–444. https://doi.org/10.1080/10739149.2015.1127256

[11] Saha, K., Agasti, S. S., Kim, C., Li, X., & Rotello, V. M. (2012). Gold Nanoparticles in Chemical and Biological Sensing. *Chemical Reviews*, *112*(5), 2739–2779. https://doi.org/10.1021/cr2001178