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Introduction

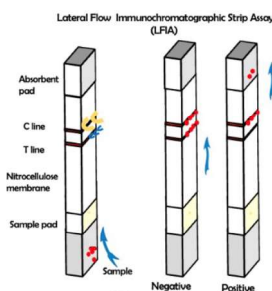
Aflatoxins are a hazardous class of fungal metabolites produced primarily by *Aspergillus* species. These species contaminate food products, posing serious health implications in humans.

Preventive measure? Rapid on-site detection using Lateral Flow Immunoassay (LFIA) strips.

How can the overall performance of LFIA be enhanced? Optimisation of antibody-nanoparticle binding conditions such as pH, NaCl, and Antibody concentration.

Aim? To determine the optimal pH, NaCl, and Ab concentration at which highly stable conjugates can be formed.

Analytical Techniques: DLS and UV-Vis were used to measure hydrodynamic size, absorbance, optical density, zeta potential, and polydispersity index.



Methodology

1 Gold NPs

Silver NPs

2 NaCl

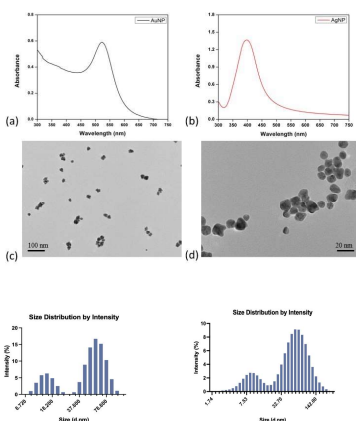
3 pH

4 Ab Concentration

(1) Synthesis of stable, monodispersed gold (Au) and silver (Ag) nanoparticles. (2) Analysis of nanoparticle aggregation behaviour in varying alkaline environments. (3) Use of K_2CO_3 to modify the colloidal solution pH during antibody attachment to the nanoparticles. (4) Stability study of nanoparticles at varying antibody concentrations under controlled pH and NaCl conditions.

Results and Discussion

Characterisation of Nanoparticles

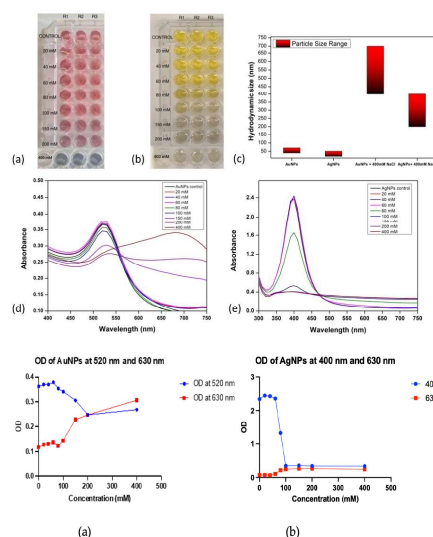


	AuNPs	AgNPs
ZP (mV)	-26.3 ± 4.6	-20.07 ± 0.5
PDI	0.209	0.564

AuNPs, synthesized using trisodium citrate, exhibited a 520 nm SPR peak, a spherical morphology, and had a hydrodynamic diameter range of 9-120 nm, indicating high stability via a high ZP.

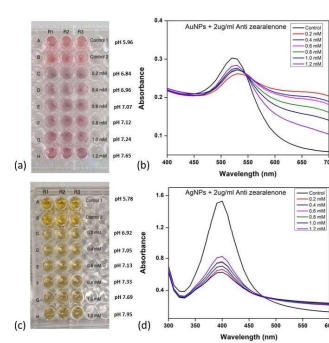
AgNPs, produced using sodium borohydride, displayed a 400 nm SPR peak, a spherical morphology, and had a hydrodynamic size range of 2.5-120 nm, confirming their stability in the colloidal solution through a high ZP.

Aggregation Response of Nanoparticles to NaCl



Both NPs behaved similarly. Onset aggregation of AuNPs and AgNPs occurred at 150 mM and 80 mM NaCl, respectively, evidenced by shift in colour and increase in hydrodynamic size. Absorbance peaks shifted to longer wavelengths which is consistent with OD pattern. This is due to the stronger interaction of the Na^+ and Cl^- ions at higher NaCl concentrations with the surface charge of NPs, resulting in changes in their physical and chemical properties, ultimately leading to the formation of large aggregates.

Optimisation of pH Conditions for the Conjugation of Nanoparticles



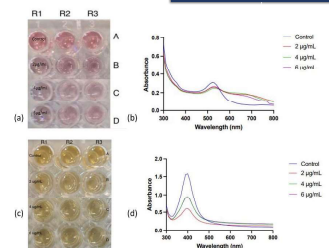
The aim was to determine the optimum K_2CO_3 concentration at which the NP-Ab conjugates exhibit high stability.

Suboptimal NaCl concentration was added to validate the conjugation process, ensuring the correct orientation and coverage of NP surfaces with antibodies.

Lower pH environment resulted in unsuccessful conjugation of NP-Ab and significantly low SPR peak intensities.

Optimal K_2CO_3 concentration was 1.2 mM (pH 7.65) for both AuNPs and AgNPs. Evidenced by minimal colour change and SPR peaks closely mirroring control peaks at 1.2 mM.

Antibody Study



The goal was to establish the optimum antibody concentration to improve nanoparticle surface binding and, consequently, enhance signal output and shelf-life.

Highly negative ZP of AuNP-Ab and AgNP-Ab conjugates were achieved at 4 and 6 $\mu\text{g/mL}$ Ab concentration, respectively, signifying substantial stability and an efficient conjugation process.

Ab Concentration ($\mu\text{g/mL}$)	ZP (mV)
Control	-30.9
2	-0.529 ± 0.54
4	-30.53 ± 1.80

Ab Concentration ($\mu\text{g/mL}$)	ZP (mV)
Control	-31.5
2	0.329 ± 0.16
4	0.403 ± 0.639
6	-36 ± 2.11

Conclusion

- AuNPs and AgNPs were sensitive to high NaCl concentrations, aggregating at 150 mM and 80 mM, respectively, as indicated by color change, SPR shifts, and surface charge changes.
- Optimum K_2CO_3 concentration for both NPs was 1.2 mM.
- Stable and maximum antibody conjugation occurred at 4 $\mu\text{g/mL}$ for AuNPs and 6 $\mu\text{g/mL}$ for AgNPs under controlled pH and salt conditions.
- These stable conjugates can enhance LFIA strip performance, sensitivity, and shelf life, ensuring the accurate detection of aflatoxins.

References

- Zheng, T., Finn, C., Parrett, C. J., Dhume, K., Hwang, J. H., Sidhom, D., Strutt, T. M., Li Sip, Y. Y., McKinstry, K. K., & Huo, Q. (2017). A Rapid Blood Test To Determine the Active Status and Duration of Acute Viral Infection. *ACS Infectious Diseases*, 3(11), 866–873. <https://doi.org/10.1021/ACSINFEC.7B00137>
- Mwakyali, S. E., Ding, X., Ming, Z., Tong, W., Zhang, Q., & Li, P. (2019). Recent development of aflatoxin contamination biocontrol in agricultural products. *Biological Control*, 128, 31–39. <https://doi.org/10.1016/J.BIOCONTROL.2018.09.012>