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Original Article

# Colorimetric Detection of Biomolecules Using Paper-Embedded Silver Nanoparticles

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***Abstract***

*This study presents a low-cost and effective colorimetric paper-based sensor for the selective detection of biomolecules using silver nanoparticles (AgNPs). Silver nanoparticles were synthesized through the chemical reduction of silver nitrate using trisodium citrate and sodium borohydride as reducing and stabilizing agents. The resulting colloidal AgNPs exhibited a characteristic yellow colour due to surface plasmon resonance (SPR) near 410 nm. The AgNPs were immobilized onto Whatman filter paper No. 1 to fabricate a simple, portable sensing platform. The detection principle is based on visible colour changes that occur upon interaction between AgNPs and analytes such as riboflavin and tannic acid, enabling rapid qualitative and quantitative assessment. UV-Vis spectrophotometry was used to correlate spectral variations with colorimetric responses. The proposed sensor provides a rapid, reproducible, and economical method for biomolecule detection without requiring advanced instrumentation, making it suitable for environmental, food-quality, and biochemical applications.*

***Keywords:*** *Silver nanoparticles, Colorimetric detection, Paper-based sensor, Biomolecules, UV-Vis spectroscopy*

## Introduction

Nanoscience and nanotechnology are modern research frontiers, yet nanoscale materials have been used for centuries. For example, gold and silver nanoparticles were unknowingly employed in medieval stained-glass windows to produce vivid colours. Today, nanotechnology enables the precise manipulation of matter at extremely small scales to achieve desirable optical, chemical, and mechanical properties, resulting in applications across diverse technological and industrial sectors.[4]

Among metallic nanomaterials, **silver nanoparticles (AgNPs)** possess exceptional optical properties due to their ability to strongly absorb and scatter light. This phenomenon arises from **Localized Surface Plasmon Resonance (LSPR)** [16] the collective oscillation of conduction electrons stimulated by incident light when nanoparticle dimensions are smaller than the wavelength of the light source. AgNPs also exhibit enhanced chemical reactivity because their reduced particle size results in a significantly larger surface-to-volume ratio. As particle size decreases, a greater proportion of atoms reside at or near the surface, increasing the material's reactivity compared to the bulk form. [20] Consequently, materials that are inert in bulk often become highly reactive at the nanoscale.

**The objective of this study is to investigate the colorimetric and optical detection of specific biomolecules riboflavin and tannic acid using AgNPs**.

These biomolecules were selected after preliminary screening due to their strong interaction with AgNPs and clear optical signatures. Riboflavin (vitamin $B_2$), chemically known as 7,8-dimethyl-10-ribityl-isoalloxazine ($C_{17}H_{20}N_4O_6$), is a water-soluble vitamin abundant in dairy by-products such as whey and in egg whites. It appears as orange-yellow crystals and shows limited solubility in water. Tannic acid, a plant-derived polyphenol, is known for its strong reducing and metal-binding properties, making it an excellent candidate for colorimetric sensing.



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Interactions between these biomolecules and AgNPs were studied primarily through **UV–Vis spectroscopy**, supported by visual colorimetric observations.

The objective of this prospective study to probe the colorimetric and optical detection of biomolecules by AgNPs, eg., riboflavin, rutin, tannic acid, etc. is because of its highly sensitivity and selectivity. Vitamin B2 also known as Riboflavin (RF) is one of the B vitamins. RF (7,8-dimethyl-10-ribityl-isoalloxazine) ($C_{17}H_{20}N_4O_6$, see Fig.1) is a water-soluble vitamin present in a wide variety of foods, abundantly found in whey (the watery part of milk) and in egg white. It can be crystallized as orange-yellow crystals, and in its pure form is poorly soluble in water. In view of this the study between RF and AgNPs by UV-Vis spectroscopy.

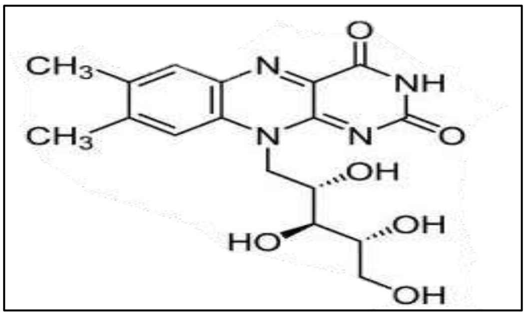


Fig.1. Stick-type (2D) molecular structure of riboflavin

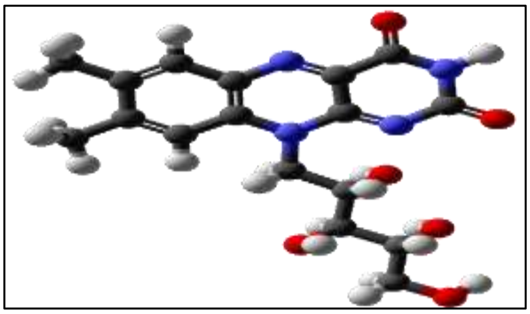

Fig.2. 3D molecular structure of riboflavin

Tannic acid (1,2,3,4,6-penta-*O*-{3,4-dihydroxy-5-[(3,4,5-trihydroxybenzoyl) oxy] benzoyl}-D-glucopyranose, is a form of tannin and a type of polyphenol. Tannic acid is often given as ($C_{76}H_{52}O_{46}$, see Fig.3), which conforms with decagalloyl glucose, it is a mixture of polygalloyl glucoses or polygalloyl quinic acid esters with the number of galloyl moieties per molecule ranging from 2 up to 12 depending on source of plant it is extracted from.

The major purpose of the present evaluation is to investigate the interaction between AgNPs and three chosen biomolecules (riboflavin, rutin and tannic acid) in certain concentration range through absorption spectra and paper-based sensor.

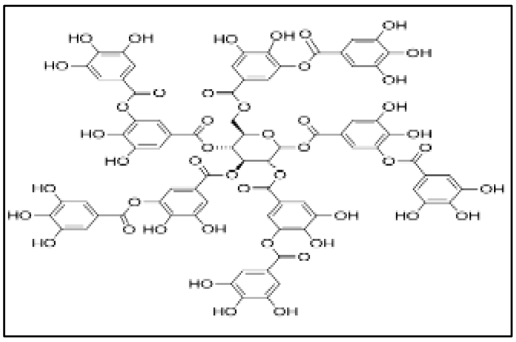

Fig.3. Stick-type (2D) molecular structure of riboflavin

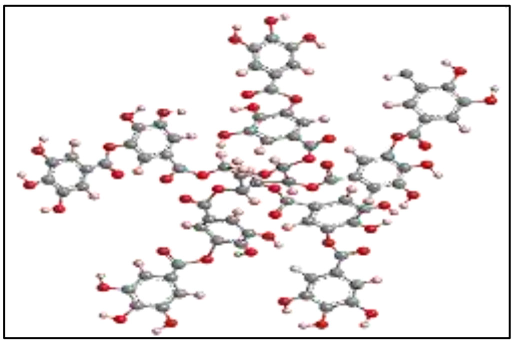

Fig.4. 3D molecular structure of riboflavin

**Experimental Section**

**Materials**

All reagents used in this work were of analytical grade. Silver nitrate ($AgNO_3$, purity 99.99%, Sigma-Aldrich), tri-sodium citrate ($C_6H_5Na_3O_7{\cdot}2H_2O$, purity 99-100.5%, Pallav), sodium borohydride ($NaBH_4$, purity 97.0%, Pallav), tannic acid ($C_{76}H_{52}O_{46}$), rutin($C_{14}H_{30}O_{16}$), riboflavin($C_{17}H_{20}N_4O_6$). Whatman filter paper (No.1). All solutions of reacting materials were prepared using high-performance liquid chromatography (HPLC) water.

**Apparatus**

Absorption spectra of the synthesized AgNPs and different biomolecules were measured using a UV-Vis spectrophotometer (Shimadzu 148). An android smartphone with 108MP resolution was used to record image of paper substrate containing AgNPs and different biomolecules.

**Synthesis of silver nanoparticle**

In this analysis, silver nitrate of 0.1M and tri-sodium citrate (0.0005N) was used as starting material for preparation of silver nanoparticles [7]. The silver colloid was prepared by using chemical reduction method. All solutions of reacting samples were prepared in HPLC water. In typical experiment firstly 0.0735g of tri-sodium citrate was dissolved in 5ml of water ensuring it to be a homogenous solution later to which 45ml of water was added making the total volume of 50ml[4]. To this solution 0.25ml(250µL) was added through micropipette. The reaction mixture was stirred vigorously using a magnetic stirrer at room temperature, after 5-6mins 1-2drops of sodium borohydride of 0.1N was pipetted precisely. The reduction process terminates with the colour change from transparent to bright yellow, which showed the formation of AgNPs after which the solution was stirred(4-5mins) at room temperature and the stir bar was removed. The nanoparticle solution was stored in refrigerator (9±1) for further stability study. [40]

This is consistent with mechanism were a part of tri-sodium citrate is used to reduce $Ag^+$ to $Ag^0$ and the remaining sodium citrate ions stabilize the particles acting as capping agent inhibiting small particles to aggregate into bigger ones.

$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2\uparrow$

Whereas sodium borohydride was drawn down to reduce the ionic silver resulting in aggregation of insoluble atoms forming clusters growing to reach a nanoscale size.

$AgNO_3 + NaBH_4 \rightarrow Ag + {}^1/_2H_2 + {}^1/_2B_2H_6 + NaNO_3$

The treatment combinations for synthesis of AgNPs is depicted in Fig.5. The colloidal solution of silver nanoparticles was characterized by using UV-Visible spectroscopy. [18]

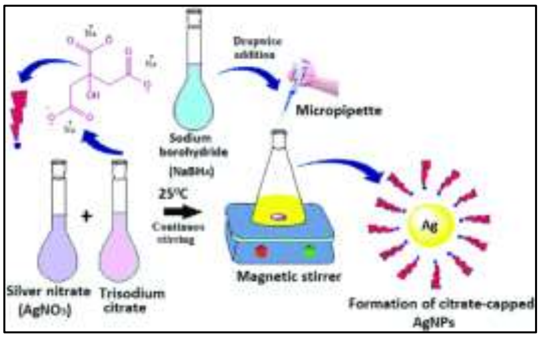


Fig.5. Schematic representation of synthesis of silver nanoparticle by TSC.


Fig.6. Silver nanoparticle structure created in 3D-Chem software.

**Sample Preparation:** Selected biomolecules (Creatinine, Nicotinic acid, Riboflavin, Rutin, L-Tryptophan and Tannic acid) of different concentrations were assembled -

1000ppm(1ml)- 1mg biomolecule + 1ml water (HPLC)

500ppm(1ml)- 0.5ml from 1000ppm and made up to 1ml

100ppm(1ml)- 0.2ml from 500ppm and made up to 1ml

Similarly, 90, 80, 70….10ppm sample solutions were prepared from 100ppm solution. Sample solutions from 9-1ppm were prepared from 10ppm solution. All reaction solutions were made with HPLC water.

**Result and Discussion**

**A. Screening of different biomolecules:**

Solutions of six biomolecules-creatinine, nicotinic acid, rutin, riboflavin, tannic acid, and L-tryptophan were prepared at a concentration of 500 ppm. Equal volumes of AgNPs and each biomolecule solution (1:1 ratio) were mixed in separate vials and allowed to stand at room temperature for 5 minutes as the binding period. Distinct visual changes in the color of AgNPs were observed for three biomolecules: rutin produced a blood-red coloration, tannic acid yielded a caramel-yellow shade [15], and riboflavin induced a tangerine coloration [5], while the remaining biomolecules exhibited negligible or no change. These mixtures were subsequently subjected to UV-Vis spectrophotometric analysis to evaluate their optical responses. The corresponding spectra are presented in Fig.9.

In the second stage, 500-ppm solutions of the same biomolecules were applied individually onto AgNP-loaded Whatman filter paper [8]. The test zones were maintained at room temperature for 5 minutes, consistent with the solution-phase reaction time. The paper substrates displayed clear, visually distinguishable color changes only for rutin, riboflavin, and tannic acid, confirming their strong interaction with the AgNPs. The screening outcome on the paper substrate based solely on naked-eye visual observation is shown in Fig. 8. [1]

Solutions of different biomolecules- Creatinine, Nicotinic acid, Rutin, Riboflavin, Tannic acid and L-Tryptophan were made of concentration 500ppm. Sample solutions holding AgNPs and above biomolecules in ratio of 1:1 were poured in separate vials and placed at room temperature for 5mins (binding time) after which an obvious colour change of AgNPs from yellow to blood red was shown by rutin, caramel yellow by tannic acid and tangerine by riboflavin. The mixture was subjected to spectrum analysis. Based on these pronounced responses, further detailed studies were carried out specifically for tannic acid and riboflavin. The results are shown in Fig.10 & 11.


Fig.7. (a) Plain biomolecule solutions (500 ppm) showing their inherent colour in the absence of AgNPs. (b) The same biomolecule solutions after mixing with AgNPs in a 1:1 ratio and incubating for 5 minutes. Distinct colour changes are observed only for rutin (blood red), tannic acid (caramel yellow), and riboflavin (tangerine), indicating strong

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interaction with AgNPs, while the remaining biomolecules show negligible change.

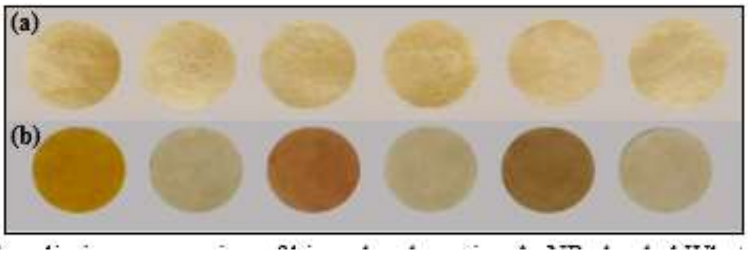


Fig.8. Paper-based preliminary screening of biomolecules using AgNPs-loaded Whatman filter paper(1).

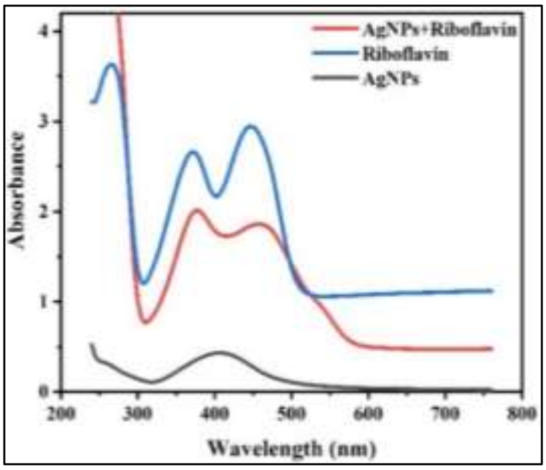


Fig.9. Rutin, tannic acid, and riboflavin exhibit significant spectral shifts and absorbance variations, confirming selective interaction with AgNPs

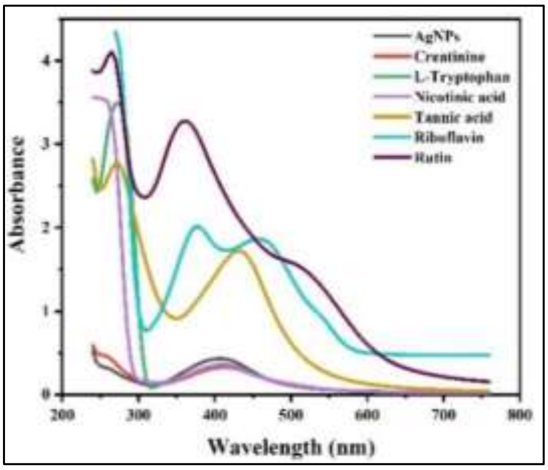


Fig.10. Absorbnace spectra of AgNPS+Riboflavin.

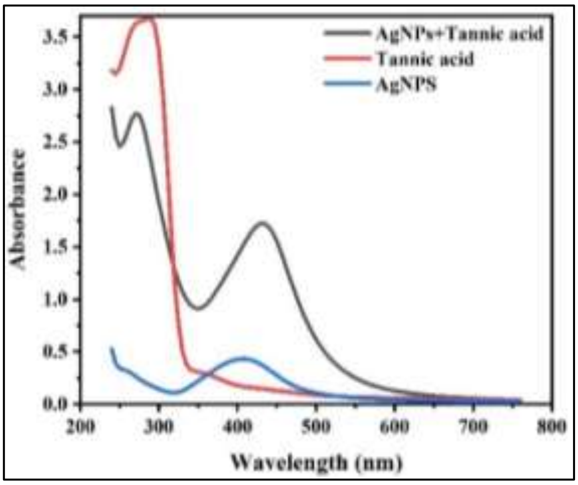


Fig.11. Absorbnace spectra of AgNPS+Tannic acid.

**B. Optimization**

**(i) pH-** The pH of sample solution is an extremely crucial parameter to for detection of desired molecule using colorimetric detection. Henceforth, 100ppm of each biomolecule (Riboflavin & Tannic acid) solution were composed of Ph (2,4,6,8,10,12) by addition of NaOH and HCl accordingly. The colour change of NPs after 5 mins was assessed through absorption spectrum.

Outcome-

- The pH range of 6-8 showed better results for sensing of tannic acid due to favouring of chemical interaction between analyte and NPs, shown in Fig.15.[31]
- pH of 6 was obtained as favourable condition for detection of Riboflavin, shown in Fig.13.[22]

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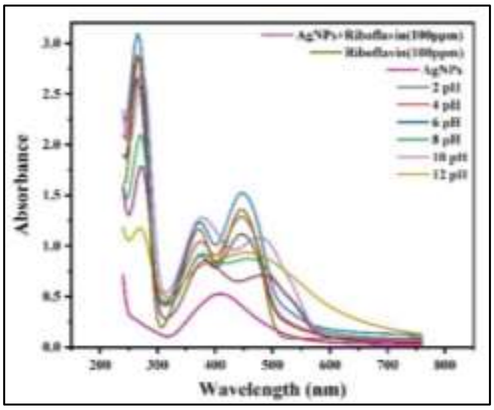



Fig.12. Maximum absorbance variation is observed at pH 6, confirming it as the optimal condition for riboflavin sensing.


Fig.13. Visual colour response of riboflavin mixed with AgNPs at different pH (2-12).

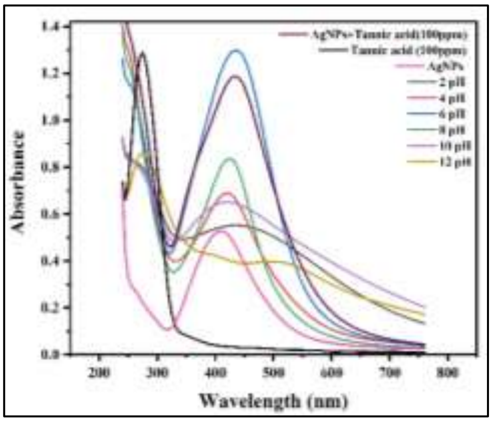



Fig.14. The spectra show enhanced aggregation-induced changes at pH 6-8, indicating optimal detection conditions.

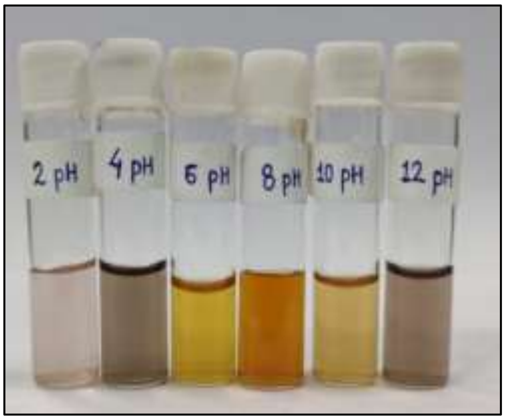
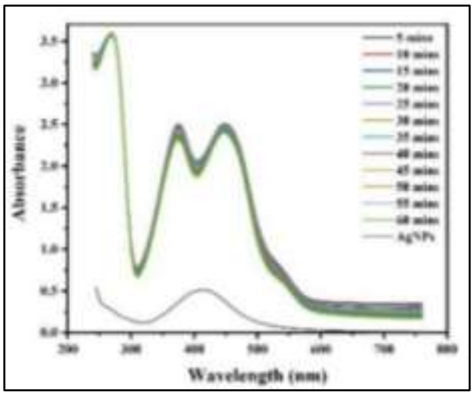



Fig.15. Visual colour response of tannic acid (100 ppm) mixed with AgNPs at different pH values (2–12).

**(ii) Reaction time-** The influence of reaction time on the interaction of AgNPs with riboflavin and tannic acid was assessed through UV-Vis spectroscopy over a 60-minute period. Riboflavin showed minimal spectral variation, with nearly overlapping SPR peaks throughout the measured intervals, indicating weak interaction and rapid stabilization of the AgNP dispersion.[29] In contrast, tannic acid produced clear time-dependent spectral changes, including a notable red-shift and a progressive increase in SPR absorbance within the first 20-25 minutes, reflecting strong nanoparticle aggregation. Beyond this point, the spectra became steady and remained unchanged up to 60 minutes, suggesting completion of the reaction. Overall, riboflavin exhibited negligible reaction-time dependence, whereas tannic acid demonstrated distinct and rapid optical evolution before reaching equilibrium.



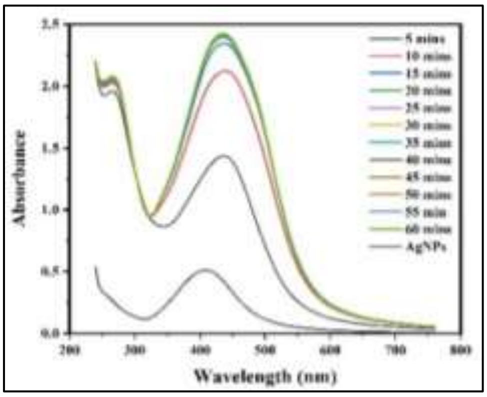



Fig.16. & 17. UV-Vis spectral changes of AgNPs in the presence of riboflavin (20 ppm) tannic acid (20 ppm) and recorded at different reaction times (5-60mins) [5,24]

The short-interval reaction-time study (2–10 minutes) revealed distinct interaction behaviors of riboflavin and tannic acid with AgNPs Riboflavin produced gradual and comparatively mild spectral changes, characterized by a slight red-shift and a modest increase in SPR absorbance over time, indicating slower surface interaction and limited nanoparticle aggregation. In contrast, tannic acid induced clear and rapid spectral evolution within the same interval, including a pronounced red-shift and a steady hyperchromic increase, reflecting strong affinity and fast aggregation of AgNPs. While tannic acid approached spectral stabilization by 8-10 minutes, riboflavin continued to show incremental changes, confirming weaker and slower interaction kinetics. Overall, tannic acid demonstrated significantly higher reactivity toward AgNPs than riboflavin in the initial reaction window.

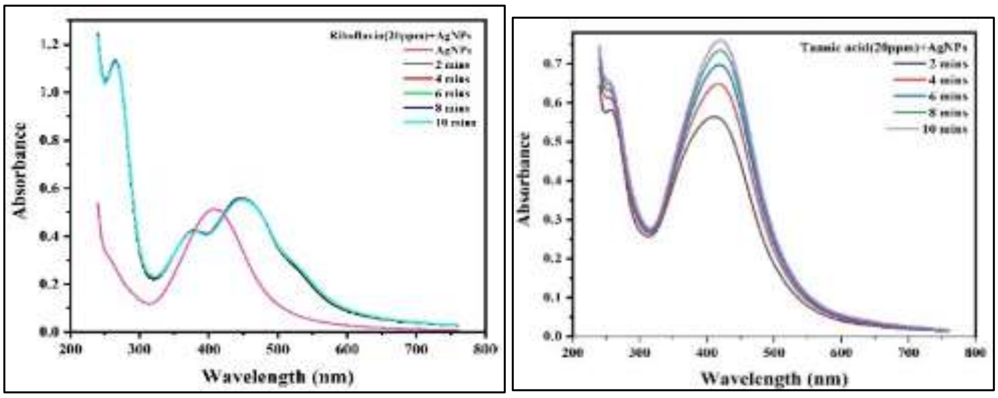



Fig.18 & 19. UV-Vis spectral changes of AgNPs in the presence of riboflavin (20 ppm) tannic acid (20 ppm) and recorded at different reaction times (2-10mins)

**(iii) Repeatability-** The above method exhibited high stability.

The relative standard deviation (RSD%) of 5 successive measurements for each of the three biomolecules are-

0.164% for Riboflavin of concentration 20ppm at 410nm

1.143% for Tannic acid of concentration 20ppm at 410nm

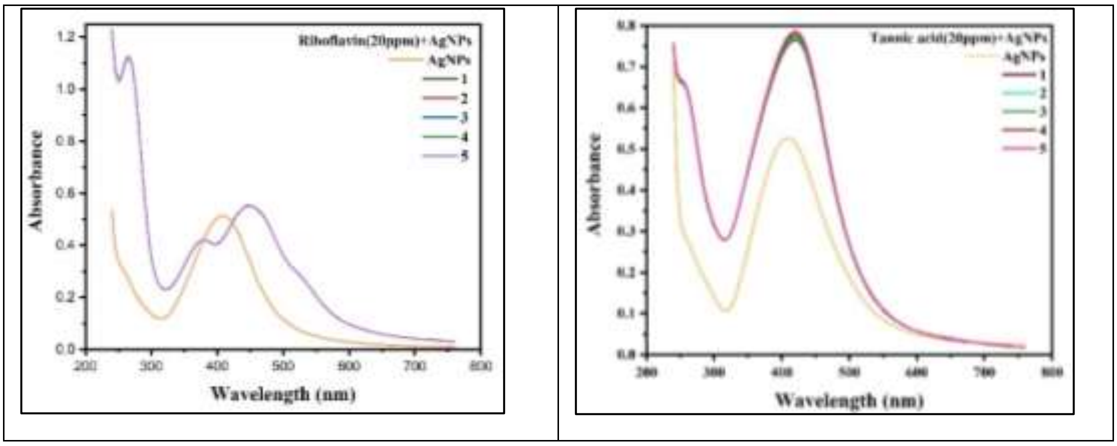



Fig.20 & 21. Overlapping spectral profiles confirm excellent repeatability and confirm the reliability of the tannic acid-AgNPs sensing mechanism.

**Characterization**

**Characterization of AgNPs colloid solution:** UV-Vis absorption spectra was used to confirm the formation of silver nanoparticles prepared in liquid state by chemical reduction method. In present experiment, citrate capped AgNPs shows intense absorption peak also known as surface plasmon resonance at $\lambda_{max}$=410nm verifying the formation of AgNPs. It is quite sensitive to the presence of silver colloids because these nanoparticles exhibit an intense absorption peak due to the surface plasmon excitation. The absorption band in the 380nm to 450nm region is typical for silver nanoparticles. The absorbance of colloid solution of Ag nanoparticles using 0.25ml of $AgNO_3$ (0.1M) were observed by the UV-VIS spectrophotometer and the absorbance of the resultant solution were recorded which is shown in the Figure.22. The spectra exhibit a plasmon absorption band at 410nm which is the characteristic of silver nanoparticles. Using 250µL $AgNO_3$ solution to 50ml of $C_6H_5Na_3O_7 \cdot 2H_2O$ (0.005N) the absorbance was 0.519 at the wavelength 410nm. [36]

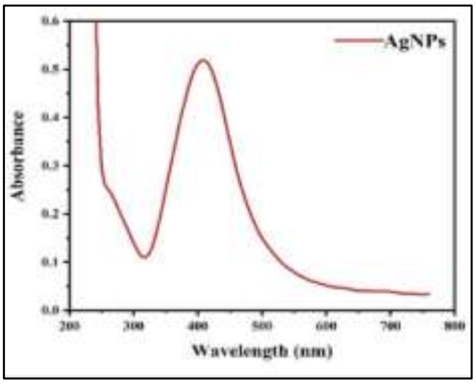



Fig.22. UV-Vis spectra and AgNPs synthesized by chemical reduction method. The plasmon peak at*410 nm confirms the formation of AgNPs.

**Spectral characterization of Biomolecules of different concentration with AgNPs:** We further evaluated the sensitivity of the colorimetric sensor towards the detection of progressed biomolecules (Riboflavin and Tannic acid). UV-Vis spectra of each biomolecule of different concentration from 1000ppm to 1ppm in presence AgNPs was studied.

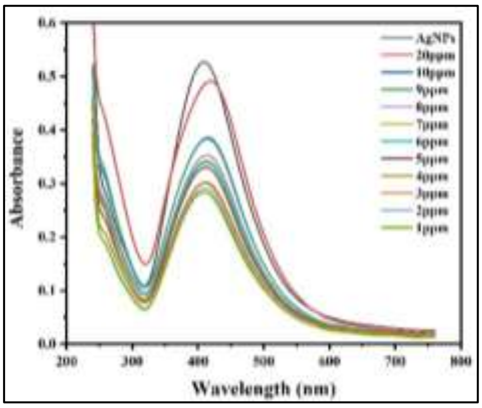



Fig.23. Change in the SPR absorption spectra resulting from the reaction of AgNPs and the different concentrations of rutin from 1 to 1000 ppm.

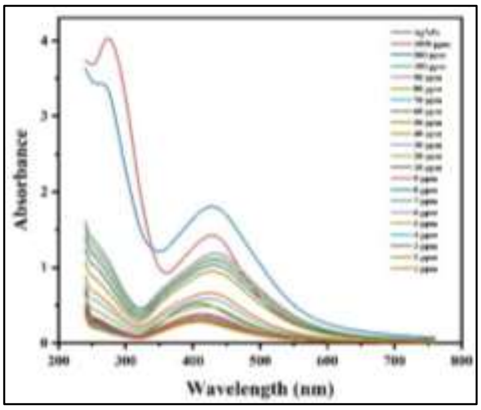



Fig.24. Change in the SPR absorption spectra resulting from the reaction of AgNPs and the different concentrations of rutin from 1 to 20 ppm

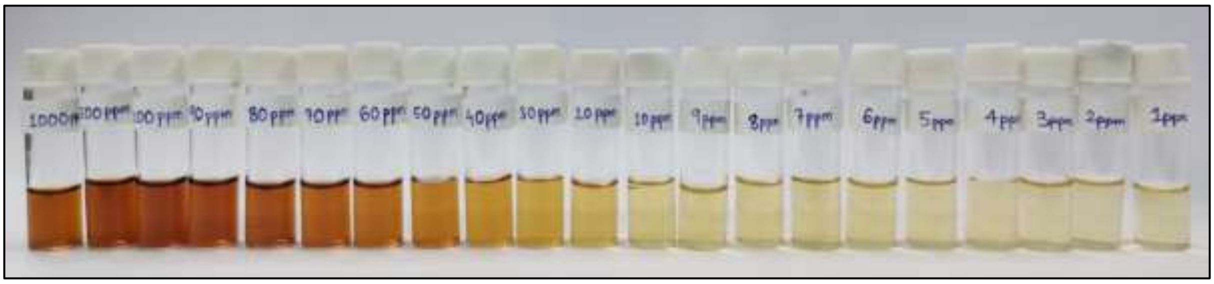


Fig.25. Serial dilutions of tannic acid (ranging from low to high ppm) mixed with AgNPs demonstrate gradual and concentration-dependent colour transitions.

Fig.26. The AgNP-coated substrate exhibits progressively intensified coloration with increasing tannic acid concentration, demonstrating strong surface reactivity and suitability for paper-based sensing.

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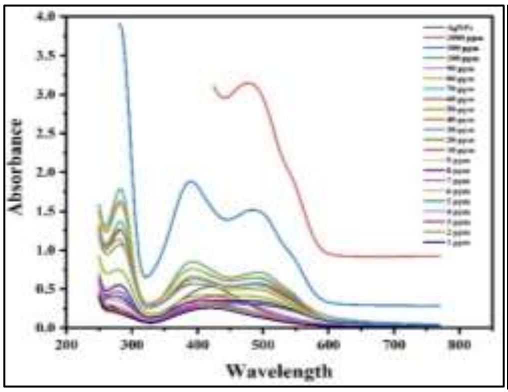


Fig.27. Change in the SPR absorption spectra resulting from the reaction of AgNPs and the different concentrations of rutin from 1 to 1000 ppm.

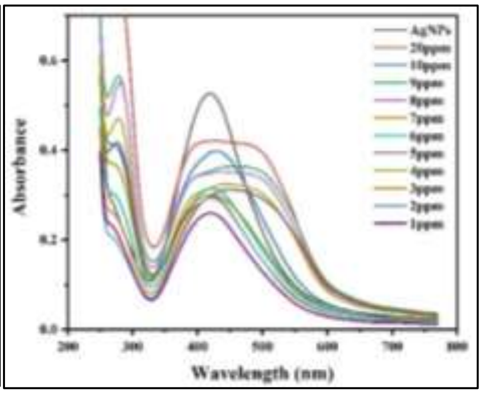


Fig.28. Change in the SPR absorption spectra resulting from the reaction of AgNPs and the different concentrations of rutin from 1 to 20 ppm

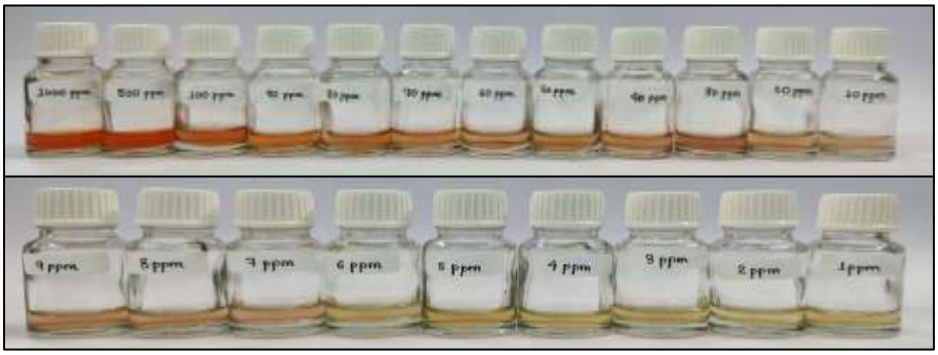

Fig.29. The samples display a visible concentration (1-1000ppm) dependent change from pale to deeper shades of tangerine as riboflavin levels increase, confirming its measurable interaction with surface-bound AgNPs.

1.143% for Tannic acid of concentration 20ppm at 410nm


Fig.30. The AgNP-coated substrate exhibits progressively intensified coloration with increasing riboflavin concentration, demonstrating strong surface reactivity and suitability for paper-based sensing.

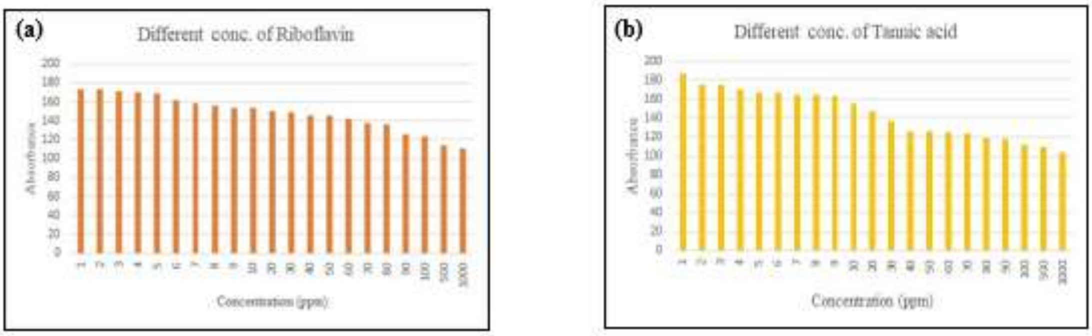


Fig.31.Bar graph representation of absorbance variation of AgNPs in the presence of different concentrations of (a) riboflavin and (b) tannic acid.

**Calculation:**

The linear relationship between absorbance variation of AgNPs and the different concentration of the three targeted biomolecules shows a good linear response in the range of 1 to 9ppm. The linear regression equation for

For Riboflavin: $y = 0.01x + 0.2558$ ($r = 0.9379$). LOD = 0.2331

For Tannic acid: $y = 0.012x + 0.2679$ ($r = 0.9732$). LOD = 2.403

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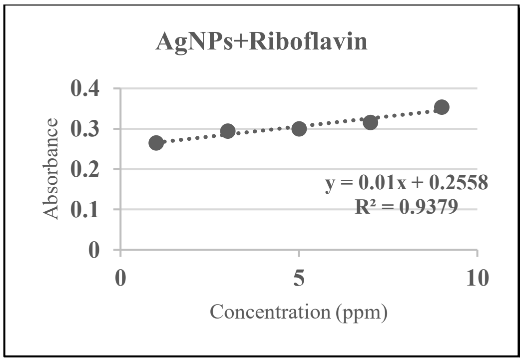


Fig.32. The plot shows a good linear relationship between absorbance change and riboflavin conc. The calculated limit of detection (LOD) for riboflavin is **0.2331 ppm**, indicating high sensitivity of the AgNP-based colorimetric system.

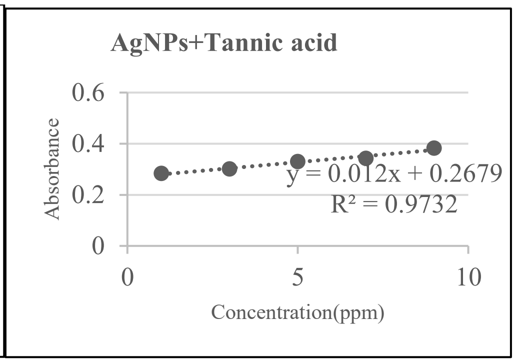


Fig.33. The calibration plot demonstrates strong linearity within the tested concentration range. AgNP sensor exhibits an LOD of **2.403 ppm** for tannic acid, confirming reliable detection performance

**Conclusion**

A simple and economical strategy for detecting biomolecules using the surface plasmon resonance (SPR) response of silver nanoparticles (AgNPs) has been successfully demonstrated. AgNPs immobilized on Whatman filter paper produced a stable paper-based colorimetric sensor capable of selective and sensitive chromogenic identification of biomolecules. The interaction of biomolecules with AgNPs led to distinct color changes due to nanoparticle aggregation. Specifically, riboflavin and tannic acid induced visible shifts from the native yellow coloration of AgNPs to characteristic shades associated with aggregated states. The detection limit was calculated to be 0.2331 for riboflavin and 2.403 for tannic acid. The corresponding spectral variations in UV-Vis analysis were used to determine the detection limits, which were found to be sufficiently low for practical analytical applications. [29,31]

The results provide a clear understanding of the optical and aggregation-based interactions between AgNPs and the selected biomolecules. Further investigations such as evaluating pH effects, concentration dependence, reaction kinetics, and competing molecular interactions can contribute to optimizing the sensor's performance and broadening its applicability in environmental, food-quality, and biochemical monitoring.

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