

SUPRAMOLECULAR AGGREGATION-DRIVEN COLORIMETRIC
DETECTION OF TETRAHYDROCANNABINOL USING β -
CYCLODEXTRIN-FUNCTIONALIZED SILVER NANOPARTICLES

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Abstract

This study presents a novel, facile approach for the colorimetric detection of tetrahydrocannabinol (THC) using β -cyclodextrin functionalized silver nanoparticles (β -CD-AgNPs), offering a rapid and label-free sensing method with visual interpretability. β -CD-AgNPs were synthesized via a green chemical reduction method, employing silver nitrate as the precursor and β -cyclodextrin as a dual-role stabilizing and functionalizing agent. Upon interaction with THC, a noticeable color shift from yellow to blue-green was observed, corresponding to nanoparticle aggregation and a red shift in localized surface plasmon resonance (LSPR). The system exhibited a linear response in the 0–35 μ M THC range with a calculated limit of detection (LOD) of 8.8 μ M and a limit of quantification (LOQ) of 26.8 μ M. Dynamic Light Scattering (DLS) and Zeta Potential (ZP) analyses were employed to evidence size augmentation (from 53.7 to 3143.6 nm) and decreasing colloidal stability (from –37.4 to –5.1 mV), respectively, with increasing THC concentrations. These physicochemical transformations validated THC-induced nanoparticle aggregation as the key sensing mechanism. Specificity studies using potential interferents such as CBN, CBD, limonene, Fe(III), and quercetin confirmed the probe's high selectivity for THC, while EDTA addition helped suppress ionic interferences. Notably, the method was extended to paper-based sensing using filter strips, enhancing field applicability. The synthesized nanoprobe were further characterized using FTIR and UV-Vis spectroscopy, corroborating successful surface modification and inclusion complexation.

INTRODUCTION

Silver nanoparticles (AgNPs) have garnered extensive interest in recent years due to their unique physicochemical properties [1], including size-dependent optical characteristics [2], high surface area [3], electrical conductivity [4], and potent antimicrobial activity [5]. These nanostructures find widespread applications [6], in biosensing [1], catalysis, drug delivery [1, 7], wound dressings [8], and water purification [1, 9]. Among these, colorimetric detection platforms based on AgNPs have emerged as particularly promising tools due to their simplicity [10], visual readability, and rapid response in detecting various analytes, including biomolecules [11], metal ions [12], and illicit drugs [13].

The optical response of AgNPs is primarily governed by their localized surface plasmon resonance (LSPR), a phenomenon where conduction electrons on the nanoparticle surface oscillate in resonance with incident light [13-16]. The LSPR band is highly sensitive to changes in particle size, shape, aggregation state, and the local dielectric environment [17]. Thus, even slight alterations in the physicochemical microenvironment around AgNPs, such as binding or interaction with target analytes, can induce measurable color changes. This property forms the basis of many LSPR-based colorimetric sensors [18].

Despite their advantages, the stability and selective reactivity of bare AgNPs in aqueous systems present persistent challenges [9]. To address these, the use of stabilizing agents is common. Stabilizers such as surfactants, polymers, polysaccharides, and oligosaccharides can effectively prevent aggregation and provide functional surfaces for interaction with target species [19]. Among these, cyclodextrins (CDs) naturally occurring cyclic oligosaccharides have gained prominence due to their unique structural features and biocompatibility [20-22].

Cyclodextrins, particularly β -cyclodextrin (β -CD), consist of seven α -D-glucopyranoside units forming a torus-like macrocyclic structure with a hydrophobic interior cavity and hydrophilic exterior rim [23]. This dual character allows β -CD

to interact with a wide range of guest molecules through inclusion complexation. Furthermore, the hydroxyl groups on β -CD rims can coordinate with metal surfaces, enabling its role not only as a stabilizer but also as a functional interface for host-guest recognition [24]. These attributes render β -CD a highly attractive candidate for the development of supramolecular nanoplateforms [25].

Incorporation of β -CD onto silver nanoparticle surfaces leads to the formation of β -CD@AgNPs [26], where the hydroxyl moieties of the CD ring chemisorb onto the silver surface, forming a protective shell that enhances colloidal stability and offers sites for specific interaction with guest molecules [27]. Several studies have confirmed such stabilizing behavior using spectroscopic and microscopic techniques. Moreover, these stabilized AgNPs exhibit retained plasmonic properties and improved dispersion in aqueous media, making them suitable for colorimetric detection applications [28].

Recent efforts have focused on exploiting β -CD-functionalized AgNPs for selective recognition and detection of molecular analytes, including toxins, drugs, and pollutants [29]. In such systems, the analyte enters the β -CD cavity, and if aggregation is induced, a plasmonic shift or color change results [30]. The extent of this spectral or visual shift can be directly correlated with analyte concentration, enabling quantitative sensing. Nevertheless, the success of these systems is dependent upon optimized host-guest interactions, nanoparticle surface design, and robust spectral correlation [31].

Among analytes of growing forensic and clinical importance is Δ^9 -tetrahydrocannabinol (THC), the primary psychoactive compound found in *Cannabis sativa* [32]. With the widespread legalization and medicinal use of cannabis, there is an urgent need for rapid, field-deployable, and cost-effective detection methods for THC. Traditional chromatographic techniques, while accurate, are expensive, require skilled personnel, and lack portability. A colorimetric assay for THC would therefore provide significant practical benefits, especially for roadside testing, point-of-

care diagnostics, and screening in clinical or security settings [33, 34].

To the best of our knowledge, no previous studies have reported a β -CD stabilized AgNP platform for the specific colorimetric detection of THC. Given the structural compatibility of THC with the β -CD cavity and its propensity to modulate the LSPR of AgNPs through induced aggregation, this study explores a novel sensing approach. Here, THC is conceptualized as a bolt-like guest molecule, fitting into the β -CD cavities on the AgNP surface, resulting in cross-linking and plasmonic coupling. The resultant aggregation causes a distinct and visible color transition, facilitating both qualitative and quantitative analysis.

The objective of this study is to develop a selective, sensitive, and rapid colorimetric sensing platform for the detection of tetrahydrocannabinol (THC) using β -cyclodextrin-functionalized silver nanoparticles (β -CD@AgNPs). Despite the widespread use of THC in medicinal, recreational, and commercial products, current detection methods such as GC-MS and HPLC, while accurate, are time-consuming, expensive, and inaccessible for point-of-care or field testing. Moreover, there is a lack of simple, low-cost, and selective colorimetric sensors capable of visually detecting THC with minimal sample processing. This study addresses that gap by utilizing the host-guest chemistry of β -cyclodextrin to induce THC-specific aggregation of silver nanoparticles, leading to a distinct colorimetric response. Through systematic characterization—including UV-Vis spectroscopy, DLS, zeta potential, and FTIR—the sensing mechanism is validated and further translated into a portable paper-strip format. The sensor's selectivity against common interferents is also explored. Ultimately, this platform aims to serve as a foundation for developing field-deployable THC screening tools, with potential future extensions toward other cannabinoids, biosensing targets, or integration into smartphone-readable diagnostics for consumer and forensic use.

Methods and Materials

Materials and Reagents

All chemicals were of analytical grade and used without further purification. β -Cyclodextrin (β -CD), silver nitrate (AgNO_3), sodium borohydride (NaBH_4), disodium ethylenediaminetetraacetate (Na_2EDTA), tetrahydrocannabinol (THC) standard, cannabidiol (CBD), cannabinol (CBN), quercetin, limonene, sodium chloride (NaCl), and ferric chloride (FeCl_3) were obtained from certified suppliers. Phosphate-buffered saline (PBS, 10 mM, pH 7.2) and Tris-HCl buffer (10 mM, pH 7.4) were used for maintaining pH during experiments. Whatman Grade 1 filter paper was used as the substrate for the paper-based sensor. All solutions were prepared using deionized water (resistivity $>18.2 \text{ M}\Omega\cdot\text{cm}$) [35].

Synthesis of β -Cyclodextrin-Functionalized Silver Nanoparticles (CD@AgNPs)

The synthesis protocol was adapted from a prior study by the research group, which previously optimized the relative ratio of AgNO_3 to β -CD to yield uniformly dispersed silver nanoparticles. The sequence of precursor and stabilizer addition was inspired by Kim et al. [36], who demonstrated that injecting a precursor solution into a preheated stabilizer matrix promotes rapid nucleation and results in smaller, monodisperse nanoparticles.

In this method, 15 mL of aqueous glucose ($0.013 \text{ mol}\cdot\text{L}^{-1}$), 15 mL of aqueous NaOH ($0.01 \text{ mol}\cdot\text{L}^{-1}$), and 30 mL of β -CD solution ($0.015 \text{ mol}\cdot\text{L}^{-1}$) were mixed under constant stirring and heated to 60°C . Once the solution reached the desired temperature, 20 mL of AgNO_3 solution ($0.01 \text{ mol}\cdot\text{L}^{-1}$) was added dropwise at a controlled rate of 1 drop per second. The mixture was stirred continuously during the addition to ensure homogeneous nucleation. After the reaction was complete, the product was dialyzed extensively against deionized water to remove unreacted species and low molecular weight byproducts.[35, 36]

The final CD@AgNPs were then centrifuged at 10,000 rpm, and after washing, the sample was kept in a desiccator to yield a dry nanoparticle

powder, which was redispersed in deionized water before use.

Characterization of CD@AgNPs

The synthesized β -cyclodextrin-functionalized silver nanoparticles (CD@AgNPs) were characterized using a variety of techniques to evaluate their structural, optical, and colloidal properties [26]. Ultraviolet-visible (UV-Vis) spectroscopy was employed to confirm the surface plasmon resonance (SPR) peak, indicating the successful synthesis of nanoparticles. Fourier transform infrared (FTIR) spectroscopy was used to identify functional groups and investigate the interaction between β -cyclodextrin and silver nanoparticles. The crystalline structure of the nanoparticles was determined by X-ray diffraction (XRD), which confirmed their crystalline nature. Morphological analysis was performed using scanning electron microscopy (SEM), revealing the shape and size of the nanoparticles. Dynamic light scattering (DLS) was used to measure the hydrodynamic size and colloidal stability, while zeta potential analysis provided insights into the surface charge and electrostatic stability of the nanoparticles. These comprehensive characterization methods ensured the successful synthesis and functionalization of the CD@AgNPs [37].

Colorimetric Detection of THC

THC standards were prepared in PBS (pH 7.2) containing 1% ethanol to ensure solubility. A 10 μ L aliquot of each standard (0, 5, 10, 20, 40, 60, 80, and 100 μ M) was applied to individual CD@AgNPs coated paper discs. After 10 minutes of incubation at room temperature, visible color changes were recorded, and photographs were taken under consistent lighting conditions using a smartphone camera. Control experiments were performed using blank samples and interferents, including CBD, CBN, quercetin, limonene, Na^+ ,

and Fe^{3+} ions, both with and without THC [38-40].

Fabrication of Paper-Based Sensor

Circular filter paper discs (1 cm in diameter) were punched from Whatman Grade 1 filter paper. Each disc was drop-cast with 10 μ L of freshly prepared CD@AgNPs dispersion and allowed to air-dry at room temperature for 15 minutes. Uniform yellow coloration indicated even nanoparticle loading. Optional hydrophobic barriers were patterned with wax printing to localize test zones and prevent lateral flow during liquid application.

Interference Mitigation Protocol

To eliminate Fe (III)-induced aggregation, samples were pretreated with 1 mM EDTA and incubated for 10 minutes before application to the paper sensor. For acidic samples, pH adjustment to 7.0 was achieved using 0.1 M NaOH or concentrated buffer solution. All detection experiments were conducted in PBS or Tris buffer to ensure consistent pH and colloidal stability.

Results and Discussion

Synthesis of CD@AgNPs

The reduction of silver nitrate (AgNO_3) was initiated by glucose, which acted as the reducing agent in an alkaline medium. The addition of β -cyclodextrin (β -CD) to the reaction mixture served as both a stabilizing agent and a structure-directing agent, preventing the aggregation of nanoparticles [41]. Upon mixing, the solution exhibited a color change from colorless to yellow, indicative of the formation of silver nanoparticles, which was further confirmed by the characteristic surface plasmon resonance (SPR) peak observed at approximately 414 nm in the UV-Vis spectrum (Fig. X). This confirms the successful formation of AgNPs and their stabilization by β -CD.

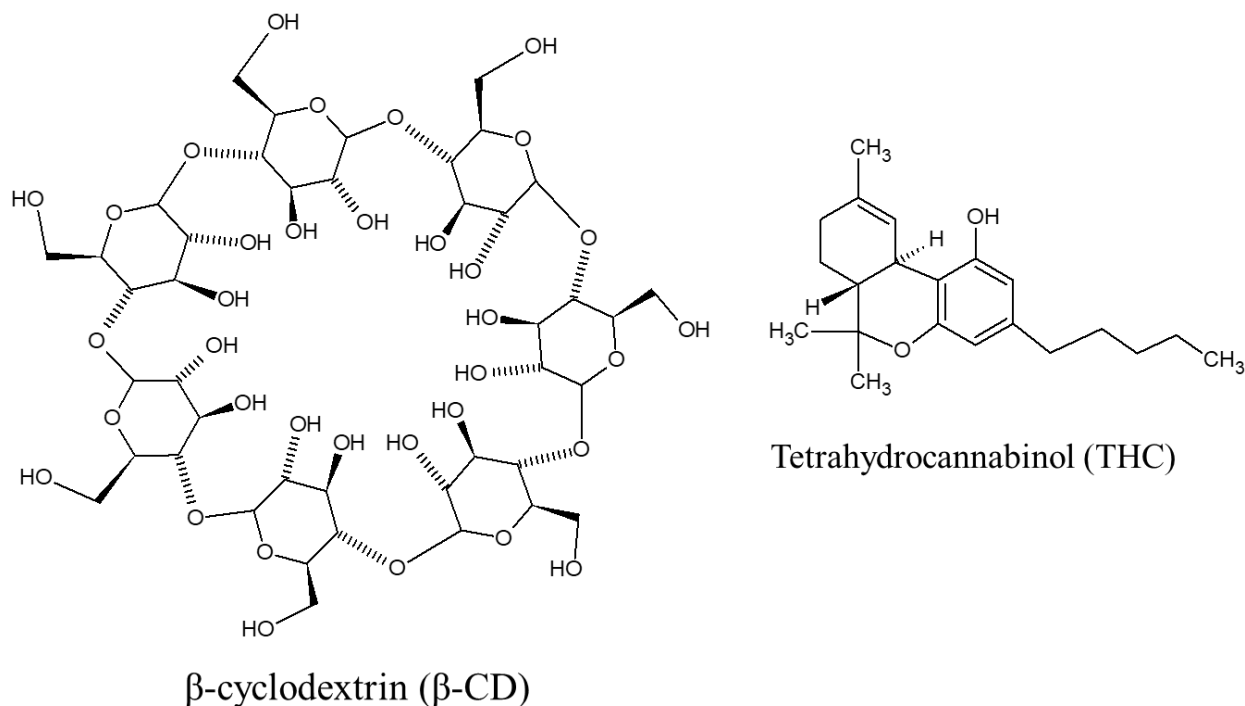


Figure 1. Structure of Cyclodextrin and Tetrahydrocannabinol

Characterization of CD@AgNPs

UV-Vis Spectroscopy

The formation of β -cyclodextrin-functionalized silver nanoparticles (CD@AgNPs) was first confirmed through UV-Vis spectroscopy, which is a widely used technique to monitor the characteristic surface plasmon resonance (SPR) of silver nanoparticles. The UV-Vis spectrum of the CD@AgNPs (Fig. X) revealed a prominent absorption peak around 414 nm. This peak is indicative of the surface plasmon resonance of the silver nanoparticles, confirming their formation [14, 15, 42].

The SPR peak is a critical feature in the UV-Vis spectra of silver nanoparticles, which occurs due to the collective oscillation of conduction electrons in response to incident light. The position and intensity of the SPR band can be influenced by various factors such as nanoparticle size, shape, and the surrounding environment. In this case, the SPR peak observed at 414 nm is consistent with the typical SPR band for spherical silver nanoparticles, suggesting that the nanoparticles synthesized in this study are predominantly

spherical and have a size range suitable for colorimetric detection applications [43].

It is important to note that the appearance of this SPR band also serves as a confirmation of successful nanoparticle synthesis. The absence of any significant shift or broadening of the peak indicates that the nanoparticles are relatively stable and well-dispersed, which is essential for ensuring reproducible results in subsequent experiments [44]. Additionally, the sharp and narrow peak further indicates the uniformity of the synthesized nanoparticles, which is crucial for their application in colorimetric detection.

The UV-Vis spectrum also provides valuable insights into the interaction between β -cyclodextrin and the silver nanoparticles. The presence of β -CD as a stabilizer is expected to influence the nanoparticle surface, which could result in a slight shift in the SPR peak. However, in this case, the peak remains consistent with typical silver nanoparticle spectra, suggesting that β -CD plays a role in stabilizing the nanoparticles without significantly altering their optical properties.

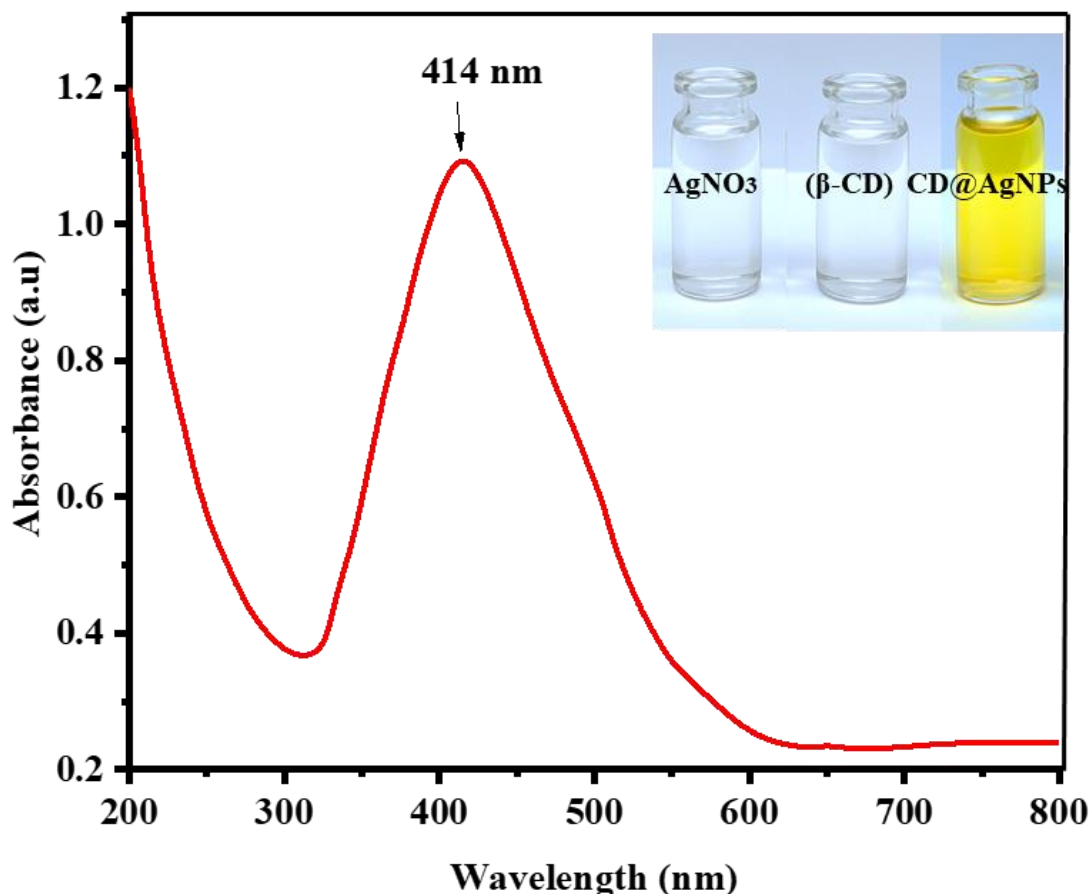


Figure 2. UV spectra of CD@AgNPs

FTIR

Fourier-transform infrared (FTIR) spectroscopy was used to confirm the successful functionalization of silver nanoparticles with β -cyclodextrin (β -CD). The FTIR spectrum of β -CD@AgNPs (Figure X) exhibits several key absorption bands characteristic of β -CD, indicating the preservation of its structural features and its attachment to the nanoparticle surface. A broad and intense absorption band at 3292.78 cm^{-1} corresponds to O-H stretching vibrations, confirming the presence of multiple surface hydroxyl groups on the β -CD structure. The peak at 2925.97 cm^{-1} is assigned to asymmetric C-H stretching vibrations of $-\text{CH}_2$ groups. The band observed at 1637.62 cm^{-1} is attributed to H-O-H bending of adsorbed water, with possible contributions from carbonyl

stretching due to trace oxidative species. In the fingerprint region, strong absorption peaks at 1155.77 cm^{-1} , 1077.81 cm^{-1} , and 1026.06 cm^{-1} are clearly observed. These are assigned to C-O-C asymmetric stretching vibrations, C-O stretching of primary alcohols, and glycosidic ring skeletal vibrations of β -CD, respectively. These peaks collectively confirm the integrity of the cyclic oligosaccharide structure. The retention of all characteristic β -CD peaks, without major shifts or disappearance, indicates that the β -CD is stably bound to the nanoparticle surface through coordination or hydrogen bonding, likely via hydroxyl groups. These findings validate the formation of β -CD@AgNPs and support their application as a functional sensing platform capable of host-guest recognition with THC molecules.

Table 1. FTIR peaks of β -CD and CD@AgNPs

Vibrational Mode	β -CD (cm ⁻¹)	β -CD@AgNPs (cm ⁻¹)	Assignment
O-H stretching	3324.16	3292.78	Slight shift
C-H asymmetric stretching (-CH ₂)	2927.26	2925.97	Minimal change
H-O-H bending / C=O stretching	1638.34	1637.62	Possible carbonyl signal overlap
C-O-C asymmetric stretching (glycosidic)	1155.78	1155.77	Intact glycosidic linkages
C-O stretching (primary alcohols)	1078.86	1077.81	Minimal shift indicates retention of CD ring structure
Ring skeletal vibrations / C-O stretching	1028.94	1026.06	Slight shift; confirms β -CD framework remains intact

XRD

The X-ray diffraction (XRD) analysis of the β -cyclodextrin-functionalized silver nanoparticles (CD@AgNPs) provides valuable insights into the structural characteristics of the nanoparticles [37]. The XRD spectrum revealed several sharp peaks corresponding to the face-centered cubic (FCC) crystal structure of silver, confirming the crystalline nature of the nanoparticles. The diffraction peaks observed at 38.6°, 44.8°, 64.8°, and 77.4° correspond to the (111), (200), (220), and (311) planes, respectively, which are characteristic of the FCC structure of silver. These peaks indicate the high crystallinity of the nanoparticles, suggesting that the functionalization with β -cyclodextrin (β -CD) did not significantly alter the crystalline structure of the silver nanoparticles.

The lattice parameters of the silver nanoparticles were calculated based on the XRD data using Bragg's law and the relationship between the interplanar spacing (d) and the Miller indices (hkl) for the FCC structure. The interplanar spacings (d) for the (111), (200), (220), and (311) planes were calculated to be 2.33 Å, 2.02 Å, 1.44 Å, and 1.23 Å, respectively. Using these d -values and the corresponding Miller indices, the lattice constant a was determined to be approximately 4.04 Å for all the planes, indicating a uniform lattice structure across the different planes. This consistency in lattice constant values suggests that

the functionalization process did not distort the nanoparticle's crystalline structure.

The average crystalline size (D) of the nanoparticles was estimated using the Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

where $K = 0.9$ is the Scherrer constant, $\lambda = 1.5406$ Å is the X-ray wavelength, β is the full width at half maximum (FWHM) of the XRD peaks, and θ is the Bragg angle. Based on the assumption of a typical FWHM value of 0.2°, the average crystalline size was found to be 45.75 nm. This value indicates that the silver nanoparticles have a relatively large crystalline structure, which is typical for nanoparticles synthesized under controlled conditions.

The dislocation density (δ), which is a measure of the number of dislocations present in the crystalline structure, was calculated using the formula:

$$\delta = \frac{1}{D^2} \quad (3)$$

where D is the crystalline size. The dislocation density for the CD@AgNPs was found to be 4.78×10^4 nm⁻², indicating that the nanoparticles have a relatively low dislocation density, which is typical for high-quality nanoparticles with well-ordered crystalline structures.

The micro strain (ϵ), which quantifies the strain present in the nanoparticles due to defects or

lattice distortions, was calculated using the formula:

$$\varepsilon = \frac{\beta}{4 \tan \theta} \quad (4)$$

where β is the FWHM in radians and θ is the Bragg angle. The average micro-strain for the CD@AgNPs was found to be 1.77×10^{-3} . This low value of micro strain suggests minimal lattice distortion and a relatively strain-free crystalline structure in the silver nanoparticles.

In conclusion, the XRD analysis confirms that the β -cyclodextrin-functionalized silver nanoparticles

retain their crystalline, face-centered cubic structure after functionalization. The calculated lattice parameters, average crystalline size, dislocation density, and micro strain indicate that the nanoparticles are of high crystallinity with a low defect density. These structural characteristics are essential for the potential applications of the CD@AgNPs, including in drug delivery, sensors, and catalysis, where stability and well-ordered structures are crucial for efficient performance.

Sample	Average crystalline size 'D' (nm)	Dislocation density (nm^{-2})	$\delta \times 10^{-3}$	Micro strain $\varepsilon \times 10^{-3}$	Degree of Crystallinity (%)
CD@AgNPs	45.75 nm	4.78		1.77	95

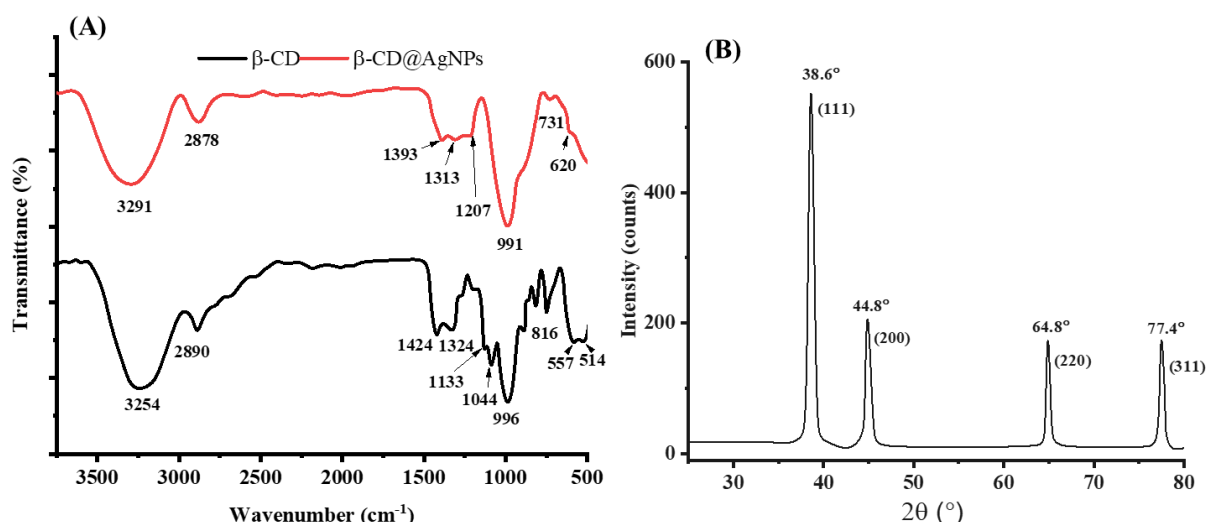


Figure 3. (A) FTIR spectra of Cyclodextrin and CD@AgNPs, and (B) XRD Spectra of CD@AgNPs

SEM

Scanning Electron Microscopy (SEM) was performed to investigate the surface morphology and particle dispersion of β -cyclodextrin-functionalized silver nanoparticles (CD@AgNPs). The SEM micrographs revealed nanoparticles with predominantly spherical to quasi-spherical morphology, consistent with typical AgNPs synthesized via reduction methods. At a lower magnification (1,000 $\times$), the nanoparticles were observed to be evenly distributed across the substrate surface with mild clustering, which is

attributed to drying-induced aggregation during sample preparation rather than intrinsic instability. At higher magnification (10,000 $\times$), the individual nanoparticles became more distinguishable, revealing uniform particle sizes in the nanometer range, broadly consistent with the crystallite size (45–50 nm) previously calculated from XRD data. The presence of small aggregates and the clear boundaries of individual particles confirm the partial stabilization conferred by the β -cyclodextrin corona, which provides steric hindrance and minimizes extensive

agglomeration. The lack of irregularly shaped particles or large-scale fusion indicates successful control over the nucleation and growth process during synthesis. These SEM observations complement the optical and DLS data, collectively confirming the formation of a monodispersed, crystalline, and functionalized AgNPs system suitable for colorimetric detection applications. Overall, the SEM images confirm that the β -cyclodextrin-functionalized silver nanoparticles

are well-dispersed, nanometer-sized, and morphologically uniform, which is essential for consistent behavior in applications such as antimicrobial activity or drug delivery. The microstructural features observed are in good agreement with the crystallite size and high crystallinity previously confirmed via XRD analysis.

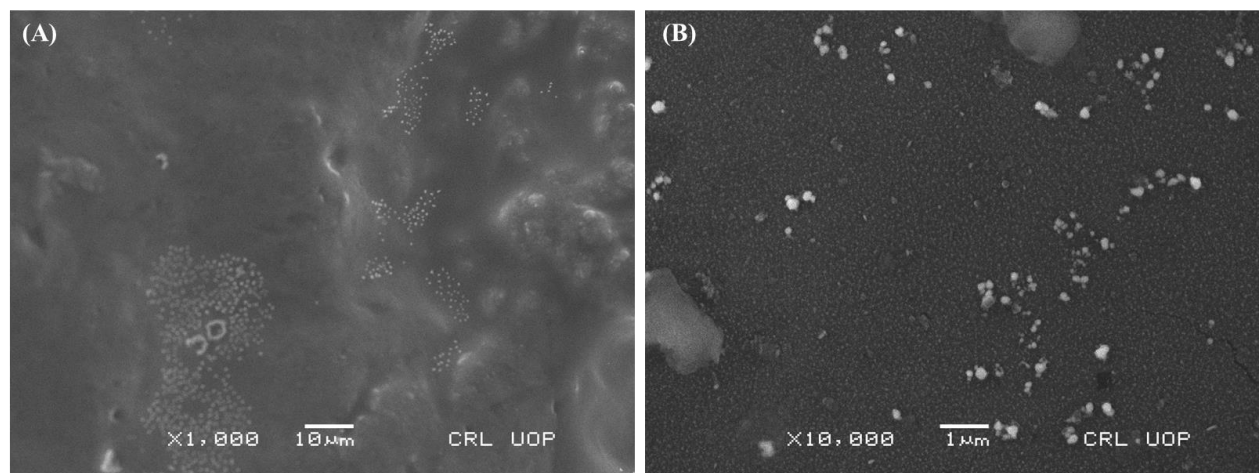


Figure 4. (A-B) SEM images of CD@AgNPs

Selective Colorimetric Detection using CD@AgNPs

The colorimetric response of β -cyclodextrin-functionalized silver nanoparticles (CD@AgNPs) was investigated in the presence of tetrahydrocannabinol (THC) and a range of potentially interfering substances to assess selectivity and application potential. UV-Vis spectral analysis revealed that pristine CD@AgNPs exhibited a sharp localized surface plasmon resonance (LSPR) band centered at 414 nm, indicating well-dispersed, stable nanoparticles. Upon addition of THC (40 μ M), a significant red-shift in the LSPR peak was observed, moving to approximately 675 nm, along with a concurrent reduction in absorbance at the original peak. This dramatic shift is attributed to aggregation caused by host-guest interactions between THC and β -cyclodextrin cavities on the nanoparticle surface, leading to reduced surface charge and particle bridging. Visually, this resulted in a distinct color

change from yellow to bluish-gray, indicating a strong and specific interaction.

To further evaluate selectivity, several analogs and common interferents—namely CBN, CBD, quercetin, limonene, NaCl, Fe(III), and acidic pH—were tested under identical conditions. Both CBN and CBD, despite structural similarities to THC, caused only minor spectral changes and retained the yellow color of the colloid, suggesting lower affinity or steric mismatch with the CD cavity. Quercetin induced slight spectral broadening, possibly due to π - π interactions, but did not cause aggregation. Limonene exhibited no observable spectral or color change, further confirming that simple hydrophobic interactions alone are insufficient to trigger the aggregation seen with THC. Inorganic ions such as NaCl and Fe(III) exhibited weak perturbations, with Fe(III) slightly diminishing absorbance via oxidative destabilization, yet neither caused the pronounced red-shift or visible color change associated with

THC. Acidic pH conditions ($\text{pH} < 5$) led to a mild decrease in LSPR intensity and some instability, likely due to protonation of hydroxyl groups on β -CD and reduced electrostatic repulsion, but again without the distinctive aggregation response.

Quantitative comparison using the observed LSPR red-shift ($\Delta\lambda$) clearly showed that THC produced the largest spectral change (approximately 261 nm), far exceeding any other tested compound. All other analytes resulted in $\Delta\lambda$ values below 60 nm, demonstrating excellent selectivity. This highly specific response is a consequence of the unique molecular fit of THC

within the β -cyclodextrin cavity, combined with its ability to mediate interparticle bridging and neutralize surface charge, ultimately leading to detectable aggregation. The visible color transition, together with the distinct spectral shift, underscores the strength of this CD@AgNP-based system for selective, rapid, and label-free detection of THC. These findings suggest that the platform holds strong potential for real-world sensing applications in complex matrices such as food, cosmetics, or biological fluids, where high specificity is essential.

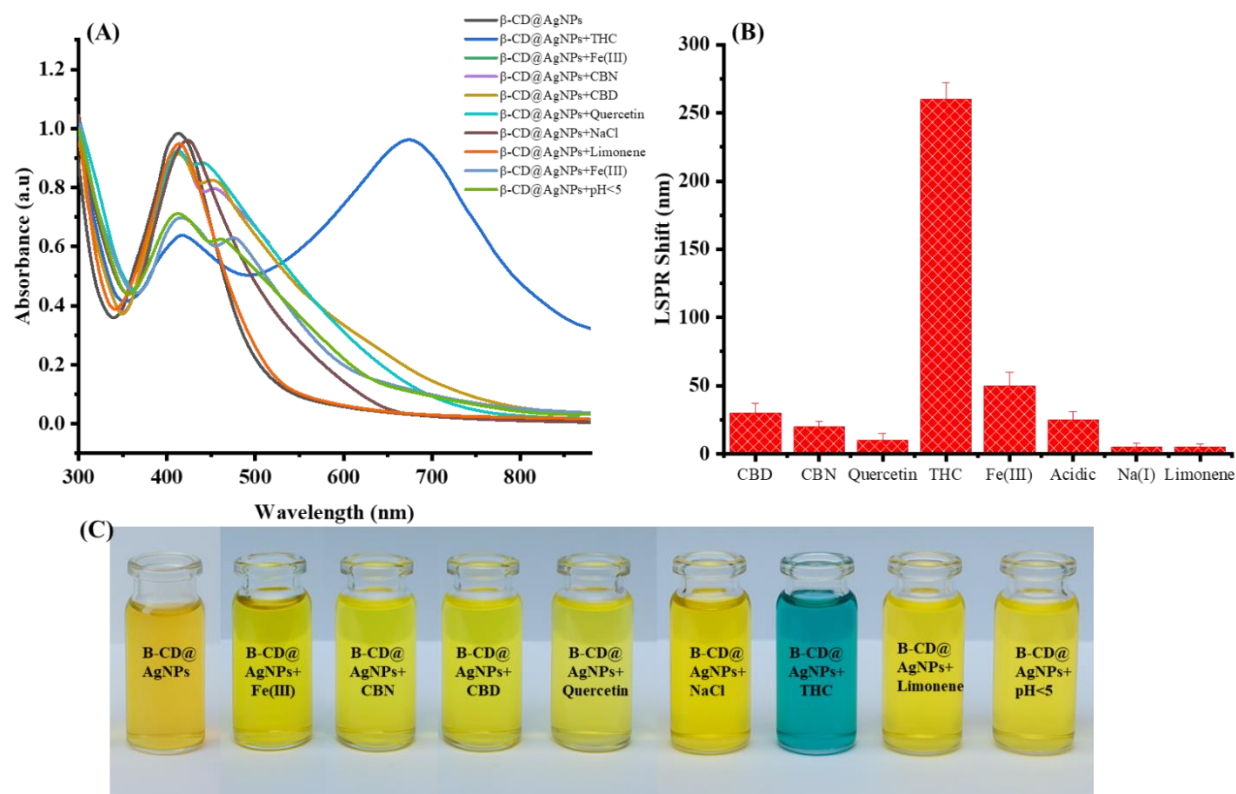


Figure 5. (A) UV-Vis absorption spectra showing colorimetric response, (B) Bar graph of absorbance of the CD@AgNPs system to THC, compared to other tested species, and (C) Visual observation of colorimetric response.

Selective Detection of THC using CD@AgNPs

To evaluate the concentration-dependent response of the CD@AgNP-based sensor, a systematic study was conducted by exposing the nanoparticles to increasing concentrations of THC ranging from 0 to 35 μM . The UV-Vis

spectra revealed a gradual and pronounced red-shift in the LSPR peak with increasing THC levels. Initially, the unmodified CD@AgNPs exhibited a sharp absorption maximum at 414 nm, indicative of stable, monodispersed nanoparticles. As THC concentration increased, a progressive decrease in

absorbance at 414 nm was observed, accompanied by the emergence and growth of a secondary peak between 600–700 nm, consistent with nanoparticle aggregation driven by THC binding. This red-shift is attributed to the inclusion of THC molecules into the hydrophobic cavities of β -cyclodextrin, which leads to a reduction in electrostatic repulsion and promotes interparticle aggregation through surface charge neutralization and molecular bridging.

Visually, these spectral changes were corroborated by a clear and progressive color transformation of the nanoparticle solution, shifting from yellow at 0 μM to green and finally bluish-green at higher concentrations of THC. This transition, visible to the naked eye, offers a practical and user-friendly colorimetric readout that aligns with the recorded spectral evolution. The strong correlation between absorbance ratio (A_{675}/A_{414}) and THC concentration was plotted to construct a calibration curve, which displayed excellent linearity in the 5–35 μM range ($R^2 = 0.995$). The minimal error bars across the concentration range suggest high reproducibility and signal stability.

The LOD and LOQ were estimated from the calibration plot using the formulas $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of the blank (0 μM THC) and S is the slope of the linear fit. The calculated LOD and LOQ were approximately 8.84 μM and 26.78 μM , confirming the sensor's suitability for mid-range THC quantification in aqueous samples.

These findings confirm that the CD@AgNPs exhibit a robust and quantifiable optical response upon interaction with THC, making the system suitable for sensitive detection. The linear relationship, together with the observable color change, supports the feasibility of using this assay for both qualitative and quantitative detection of THC in field or point-of-care applications. The colorimetric shift enables easy detection without the need for sophisticated instrumentation, while the linear absorbance response enables quantification using a basic spectrophotometer. This dual capability positions the CD@AgNP-based sensor as a promising tool for rapid, visual, and accurate detection of THC in various sample matrices.

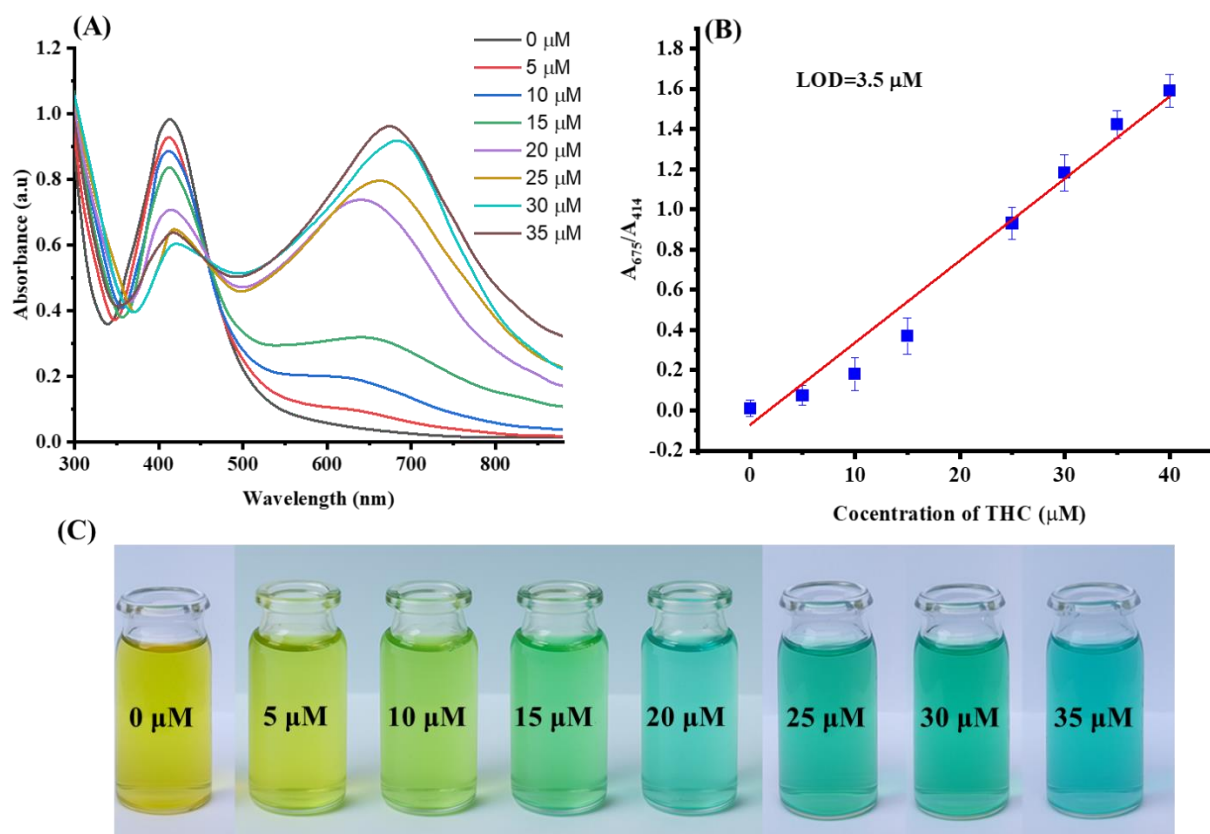


Figure 6. (A) UV-Vis absorption spectra of β -CD@AgNPs in the presence of increasing THC concentrations (0–35 μ M), (B) Linear calibration plot based on the absorbance ratio, and (C) Visual colorimetric transition of the nanoparticle solutions from yellow to greenish-blue.

Paper-Based Visualization of THC Detection

To enhance the practicality and portability of the sensing system, β -CD@AgNPs were immobilized onto filter paper strips to fabricate a paper-based colorimetric sensor. The strips were prepared by drop-casting the nanoparticle solution onto cellulose substrates and drying at room temperature. Upon exposure to varying concentrations of THC (0–35 μ M), a distinct gradient color change from yellow to greenish-blue was observed, visually correlating with solution-

phase results. This transition is attributed to THC-induced aggregation on the paper matrix, mirroring the mechanism observed in colloidal assays. The strip-based platform preserved selectivity toward THC over interferents and exhibited consistent color patterns within 5–10 minutes of sample application. These features suggest strong potential for real-time, on-site THC screening in resource-limited or non-laboratory environments.

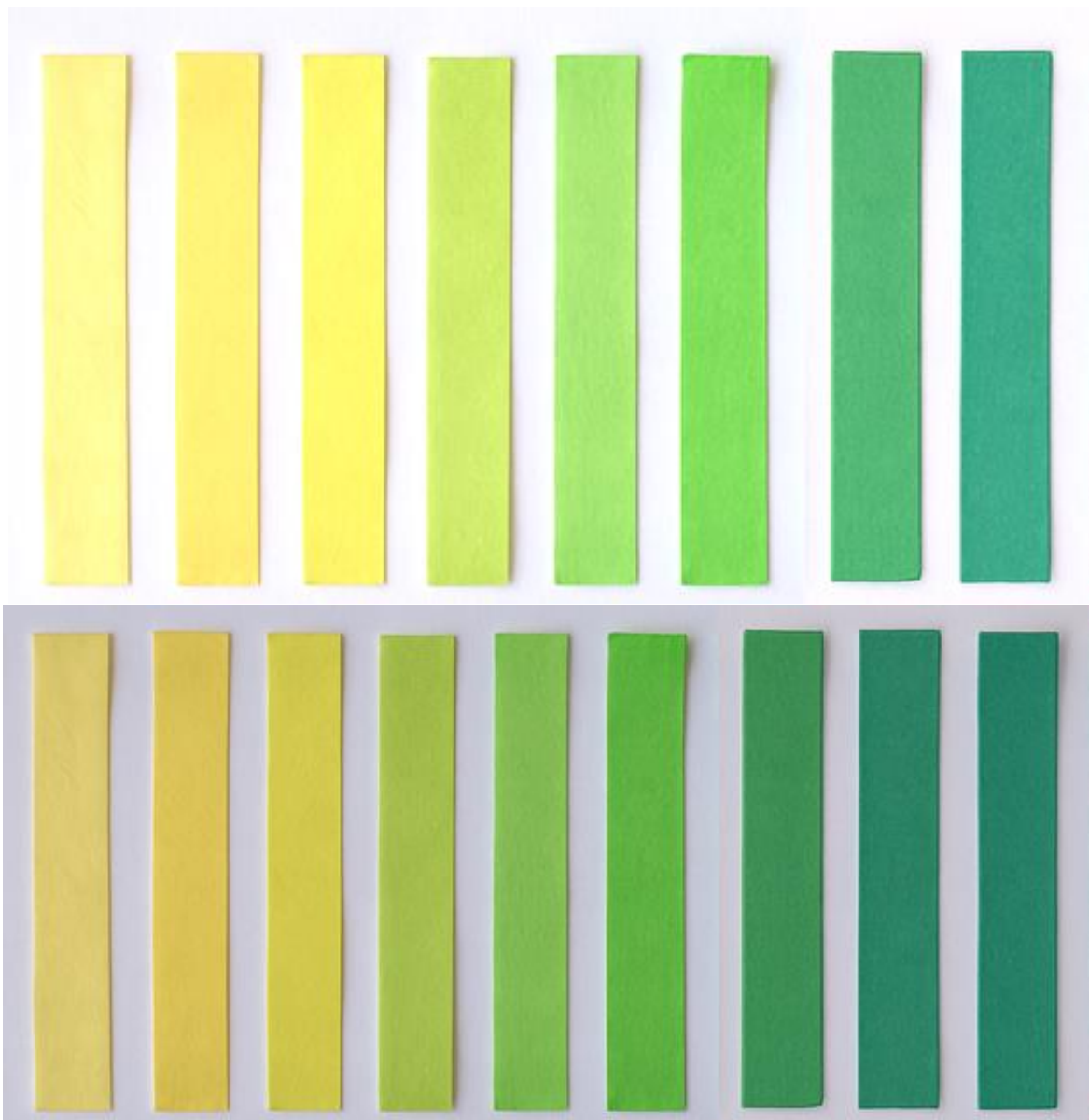


Figure 7. Colorimetric detection of THC using β -CD@AgNPs immobilized on filter paper

Conclusion

In summary, this study successfully demonstrates a novel, selective, and visually interpretable colorimetric sensing strategy for the detection of tetrahydrocannabinol (THC) based on β -cyclodextrin-functionalized silver nanoparticles (β -CD@AgNPs). The sensor leverages the host-guest inclusion properties of β -CD to trigger THC-induced aggregation of AgNPs, resulting in distinct and measurable optical changes.

Comprehensive characterization through UV-Vis spectroscopy, FTIR, DLS, and zeta potential analysis provided strong physicochemical evidence of this aggregation mechanism. The system exhibited excellent selectivity toward THC over structurally related cannabinoids and other interferents, with EDTA effectively mitigating nonspecific ionic interactions. Furthermore, the translation of the sensing assay to a paper-based platform highlights its potential for real-world

applications in point-of-care, roadside, and forensic THC screening. Overall, this work provides a cost-effective, rapid, and accessible nanomaterial-based platform that addresses key limitations of conventional analytical techniques and opens new avenues for portable cannabinoid sensing.

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