

# Dual-Mode Colorimetric Detection of Creatinine Using DTPA-Modified Silver Nanoparticles: Experimental Validation and Computational Analysis

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## Research Article

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# Abstract

Creatinine monitoring plays a crucial role in the assessment of renal function and clinical diagnostics. In this study, a dual-mode colorimetric sensing platform based on DTPA-functionalized silver nanoparticles (DTPA-AgNPs) was developed for rapid creatinine detection. The sensing mechanism relied on induced aggregation of DTPA-AgNPs through intermolecular interactions, leading to plasmon coupling and a distinct color change from yellow to brown. Density functional theory (DFT) calculations further revealed that favorable adsorption and cooperative intermolecular interactions between creatinine and DTPA-functionalized AgNPs promote nanoparticle aggregation. Response Surface Methodology (RSM) based on a Box–Behnken Design (BBD) was employed to optimize the sensing conditions, yielding optimum parameters at pH 11.81, AgNP concentration of 141.31  $\mu\text{M}$ , and detection time of 4.51 min. Under these optimized conditions, the developed sensor enabled rapid creatinine quantification through both UV–Vis spectrophotometric and smartphone-assisted RGB image analysis. The absorbance ratio ( $A_{648}/A_{397}$ ) exhibited a linear response toward creatinine concentrations ranging from 0 to 90  $\mu\text{M}$ , with limits of detection of 1.28  $\mu\text{M}$  and 0.19  $\mu\text{M}$  for UV–Vis and RGB-based measurements, respectively. The proposed sensing platform demonstrated excellent selectivity toward creatinine in the presence of potentially interfering species and was successfully applied to creatinine determination in urine samples, providing recovery values ranging from 89.7% to 109.2%.

## 1. Introduction

The detection and quantification of creatinine are important in clinical diagnostics, particularly for assessing kidney function and diagnosing renal diseases [1]. Creatinine is the most commonly used biomarker for evaluating kidney function and estimating the glomerular filtration rate [2–4]. Despite its widespread use, accurate determination of creatinine remains essential because conventional analytical methods, including Jaffé-based spectrophotometry [5], enzymatic assays [6], high-performance liquid chromatography [7], and capillary electrophoresis [8], suffer from several limitations such as interference from endogenous substances, complex sample preparation, and the requirement for sophisticated instrumentation. These drawbacks highlight the need for simple, rapid, and selective analytical methods for creatinine detection, particularly for point-of-care applications.

A range of nanomaterial-based sensing platforms, including gold nanoparticles, metal-organic frameworks, and nanozyme systems, has been investigated for creatinine detection. Despite their promising analytical performance, many of these systems still face limitations that hinder practical implementation. Gold nanoparticle-based sensors can achieve high sensitivity depending on the ligand employed, yet their detection limits vary substantially across different functionalization strategies, indicating challenges in reproducibility and standardization [9]. In addition, the relatively high cost of gold precursors further limits their widespread application. Metal–organic framework–based sensors provide large surface areas and tunable structures that enhance sensitivity, but they frequently require post-synthetic modification and multiple functional components to achieve adequate selectivity [10]. Similarly, nanozyme systems such as  $\text{Mn}_3\text{O}_4^-$  [11] or  $\text{BiFeO}_3^-$  [12] based catalysts offer enzyme-like activity but often operate within micromolar detection limits and may suffer from interference in complex biological matrices.

Among these nanomaterial-based platforms, silver nanoparticles provide a particularly attractive alternative due to their simple synthesis, strong plasmonic response, and high sensitivity to surface modification [13]. Their localized surface plasmon resonance properties are highly responsive to changes in particle size, aggregation state, and surface chemistry [14]. Interactions between analyte molecules and the nanoparticle surface can alter these plasmonic characteristics, producing measurable colour changes that enable sensitive colorimetric detection without the need for expensive instrumentation.

The performance of AgNP-based colorimetric sensors is largely governed by surface functionalization. Surface ligands not only stabilize nanoparticles but also regulate their interaction with target analytes, thereby influencing sensitivity and selectivity. Multidentate chelating ligands such as citrate [15], EDTA [16], and 2,2-thiodiacetic acid [17] have been reported to enhance sensitivity by stabilizing nanoparticles and promoting analyte-induced aggregation, with detection limits reaching the nanomolar level.

In this context, diethylenetriaminepentaacetic acid (DTPA) offers an alternative ligand for AgNP functionalization. Structurally, DTPA is analogous to EDTA but contains an extended backbone with five carboxyl groups, providing additional coordination sites and stronger multidentate binding to the silver surface [18, 19]. One carboxyl group can coordinate with the nanoparticle surface,

while the remaining negatively charged carboxylate groups contribute to electrostatic stabilization [20]. This extended multidentate structure is expected to improve nanoparticle stability while maintaining accessible functional groups for interaction with creatinine. Furthermore, the abundant carboxylate moieties of DTPA generate a negatively charged nanoparticle surface that enhances colloidal stability and dispersion. These functional groups can interact with creatinine through electrostatic attraction and hydrogen bonding [16], facilitating analyte-induced aggregation of the nanoparticles and producing a measurable colorimetric response. Such a mechanism enables sensitive detection while maintaining a simple synthesis approach. Therefore, DTPA-functionalized AgNPs represent a promising platform for developing practical, rapid, and selective colorimetric sensors for creatinine detection.

The analytical performance of the developed DTPA-AgNPs sensing system toward creatinine detection was optimized through a multivariate statistical approach using Response Surface Methodology (RSM) combined with a Box–Behnken Design (BBD). Three experimental parameters, include solution pH, detection time, and nanoparticle colloidal volume, were selected as independent variables due to their substantial influence on the plasmonic sensing response. The pH condition governs the surface charge and chemical speciation of both DTPA-functionalized AgNPs and creatinine molecules, thereby affecting the interaction mechanism and aggregation tendency of the nanoparticles. In addition, the detection time determines the progression of nanoparticle aggregation and the stabilization of the colorimetric response, while the colloidal volume influences the availability of active plasmonic nanoparticles participating in the sensing process. Optimization using conventional univariate methods is often inefficient because the influence of each parameter is evaluated separately, neglecting possible interaction effects among variables. Therefore, the RSM-BBD approach was employed to simultaneously investigate the individual and combined effects of the selected parameters while minimizing the number of experimental runs.

## 2. Experimental

### 2.1. Materials and instruments

All reagents were of analytical grade and used without further purification. Silver nitrate ( $\text{AgNO}_3$ ), diethylenetriaminepentaacetic acid (DTPA), ascorbic acid, sodium hydroxide ( $\text{NaOH}$ ), creatinine ( $\geq 98\%$ ), sodium chloride ( $\text{NaCl}$ ), glucose, uric acid, urea, glutamic acid, glycine,  $\text{Fe}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , and  $\text{NH}_4\text{Cl}$  were prepared using distilled water. UV–Vis absorption spectra were recorded using a Hitachi U-3900 spectrophotometer. Fourier transform infrared (FT-IR) spectra were obtained using a PerkinElmer spectrometer. Morphological analysis was carried out using high-resolution transmission electron microscopy (HR-TEM, JEOL JEM-2100, 200 kV). Crystalline structures were analyzed using X-ray diffraction (XRD, Rigaku SmartLab). Particle size distribution and zeta potential were measured using dynamic light scattering (DLS, Horiba SZ-100). X-ray photoelectron spectroscopy (XPS/ESCA, Shimadzu) Axis Supra<sup>+</sup> was utilized to examine the elemental composition of AgNPs.

### 2.2. Synthesis of DTPA-Capped Silver Nanoparticles

DTPA-capped silver nanoparticles (DTPA-AgNPs) were synthesised via chemical reduction using ascorbic acid as the reducing agent and DTPA as a multidentate capping ligand. In a typical procedure, DTPA (50  $\mu\text{mol}$ ) and ascorbic acid (100  $\mu\text{mol}$ ) were dissolved in an aqueous medium under continuous stirring. The solution pH was adjusted to 11 to ensure partial deprotonation of DTPA and to facilitate the reduction process. Subsequently, 0.5 mL of  $\text{AgNO}_3$  solution (equivalent to 10  $\mu\text{mol}$   $\text{Ag}^+$ ) was added dropwise while stirring continuously. The reaction mixture was maintained at room temperature for 30 minutes, during which a visible colour change from colourless to yellow was observed, indicating the formation of silver nanoparticles. The formation of DTPA-AgNPs was confirmed by UV–Vis spectroscopy, showing a characteristic surface plasmon resonance (SPR) band around 390–410 nm.

### 2.3. Colorimetric Detection of Creatinine

Creatinine standard solutions (5–90  $\mu\text{M}$ ) were prepared under alkaline conditions (pH 11) using 0.6 M  $\text{NaOH}$ . Equal volumes (1000  $\mu\text{L}$  each) of creatinine solution and DTPA-AgNP colloidal solution were mixed and incubated at room temperature. After 4.51 minutes of incubation, UV–Vis absorption spectra were recorded over the wavelength range of 300–800 nm. The analytical

signal was expressed as the absorbance ratio between the aggregation band and the initial SPR band ( $A_{648}/A_{397}$ ), which was plotted against creatinine concentration to construct a calibration curve.

## 2.4. Optimization of Experimental Variables

The experimental conditions affecting the colorimetric response of the DTPA-AgNPs sensor toward creatinine were optimised using Response Surface Methodology (RSM) based on a Box–Behnken Design (BBD). The experimental design and statistical analyses were performed using Design-Expert® 13 software. Three independent variables were selected for optimization, namely pH (A), AgNP concentration (B), and detection time (C). These parameters were chosen due to their significant influence on nanoparticle stability, analyte–nanoparticle interactions, and overall analytical performance. The absorbance response of the sensing system at the selected wavelength was used as the dependent variable. Each factor was evaluated at three coded levels (− 1, 0, and + 1). The experimental ranges were established based on preliminary investigations to ensure adequate coverage of the design space while maintaining the stability and sensitivity of the DTPA-AgNPs sensing platform. The coded and actual values of the independent variables employed in the BBD are presented in Table 1.

Table 1  
Independent variables and their coded levels employed in the Box–Behnken Design.

| Independent variable    | Symbol | −1 | 0   | + 1 |
|-------------------------|--------|----|-----|-----|
| pH                      | A      | 10 | 11  | 12  |
| AgNP concentration (μM) | B      | 50 | 102 | 154 |
| Detection time (min)    | C      | 1  | 3   | 5   |

## 2.5. Digital analysis experiments

Digital image analysis was performed by positioning the sample-containing cuvette inside a custom-designed mini studio box under controlled illumination conditions. Images were captured using an iPhone 15 at a fixed distance of 15 cm from the sample chamber to ensure consistent image acquisition. The mini studio setup (23 × 22 × 22 cm) was equipped with white background surfaces and integrated LED illumination to minimise variations caused by ambient lighting. The acquired images were subsequently processed using the ImageJ software to extract the red, green, and blue (RGB) intensity values. The obtained RGB data were then utilized to determine the Euclidean distance ( $\Delta E$ ) according to the following equation: [21]

$$\Delta d = \sqrt{(R_i - R_0)^2 + (G_i - G_0)^2 + (B_i - B_0)^2} \tag{1}$$

## 2.6. Creatinine detection in human urine samples

Creatinine detection was performed using urine samples from healthy volunteers, collected and filtered through 0.25 μm membranes. The filtered urine was then diluted 1000 times with DI water and spiked with 10, 30, and 50 μM creatinine. The sample was used as the control (0 μM). Then, 2 mL of AgNP-DTPA was added to 100 μL of spiked samples. Each sample was analyzed using a UV–Vis spectrophotometer to obtain the colorimetric response.

## 2.7. Computational Details

To provide atomistic insight into the molecular recognition mechanism responsible for the colorimetric detection of creatinine, Density Functional Theory (DFT) calculations were performed on the interaction between creatinine and DTPA-functionalized silver nanoparticles (DTPA-AgNPs). All quantum-chemical calculations were performed using the Gaussian 09 software package [22]. Several initial adsorption configurations were constructed by placing creatinine at different orientations relative to the DTPA-functionalized AgNP surface, and the lowest-energy adsorption geometry was subsequently selected for full DFT optimization.

Geometry optimizations were performed for isolated DTPA, creatinine, the silver nanoparticle model (AgNP), the DTPA-functionalized nanoparticle (DTPA-AgNP), the AgNP–creatinine complex, and the DTPA-AgNP–creatinine adsorption complex

using the M06-2X exchange-correlation functional. The M06-2X functional has been demonstrated to provide reliable descriptions of thermochemistry, non-covalent interactions, and medium-range electron correlation, making it particularly suitable for adsorption studies involving organic ligands and metal clusters [23]. The 6-311G(d,p) basis set was employed for H, C, N, and O atoms, whereas Ag atoms were represented using the LanL2DZ effective core potential (ECP) [24, 25]. Harmonic vibrational frequency calculations were subsequently performed to confirm that all optimized structures corresponded to true minima on the potential energy surface, as evidenced by the absence of imaginary frequencies.

The interaction energy describing the formation of the DTPA-functionalized silver nanoparticle was calculated according to

$$E_{\text{int}} = E_{\text{DTPA-AgNP}} - (E_{\text{DTPA}} + E_{\text{AgNP}})$$

where  $E_{\text{DTPA-AgNP}}$ ,  $E_{\text{DTPA}}$ , and  $E_{\text{AgNP}}$  denote the total electronic energies of the DTPA-functionalized silver nanoparticle, isolated DTPA molecule, and isolated silver cluster, respectively. A more negative interaction energy indicates stronger coordination between DTPA and the Ag surface and, consequently, greater nanoparticle stability.

The adsorption affinity of creatinine toward the DTPA-functionalized nanoparticle was evaluated using the adsorption energy,

$$E_{\text{ads}} = E_{\text{DTPA-AgNP/Cre}} - (E_{\text{DTPA-AgNP}} + E_{\text{Cre}}) + \delta_{\text{BSSE}}$$

where  $E_{\text{DTPA-AgNP/Cre}}$ ,  $E_{\text{DTPA-AgNP}}$ , and  $E_{\text{Cre}}$  are the electronic energies of the adsorption complex, the isolated DTPA-functionalized nanoparticle, and the isolated creatinine molecule, respectively. The basis set superposition error (BSSE) was corrected using the counterpoise method [26] to eliminate artificial stabilization arising from basis-set overlap and to obtain reliable adsorption energies.

To further elucidate the electronic origin of the sensing mechanism, frontier molecular orbital (FMO) analysis was performed to evaluate changes in the HOMO–LUMO energy gap together with related global quantum descriptors, including chemical potential, global hardness, softness, and electrophilicity. These descriptors were employed to assess the influence of DTPA functionalization and creatinine adsorption on the electronic properties, charge-transfer capability, and chemical reactivity of the silver nanoparticle.

The nature of the intermolecular interactions governing creatinine recognition was further investigated using the Quantum Theory of Atoms in Molecules (QTAIM) developed by Bader (1990). Topological analyses were performed by locating bond critical points (BCPs) and evaluating the electron density ( $\rho$ ), Laplacian of electron density ( $\nabla^2\rho$ ), kinetic energy density  $G(r)$ , potential energy density  $V(r)$ , total energy density  $H(r)$ , and the  $|V(r)|/G(r)$  ratio (Table S1). The interpretation of these topological descriptors was evaluated according to the previously proposed criteria [27], in which the combination of electron density, Laplacian, total energy density, and  $|V|/G$  ratio enables differentiation between electrostatic, hydrogen-bonding, and partially covalent interactions. In addition, Non-Covalent Interaction (NCI) analysis based on the reduced density gradient (RDG) was performed following the reported method [28] and implemented through the NCIPLOT approach to visualize attractive, van der Waals, and steric interactions within the adsorption complexes [29].

## 3. Results and discussion

### 3.1. Formation and Physicochemical Characterization of DTPA-AgNPs

The reduction of  $\text{Ag}^+$  ions by ascorbic acid proceeds spontaneously owing to the favorable redox potential difference between the  $\text{Ag}^+/\text{Ag}^0$  couple (+ 0.80 V) and the ascorbic acid/dehydroascorbic acid redox pair (+ 0.06–0.08 V). The UV–Vis spectrum of the synthesized colloid exhibited a distinct localized surface plasmon resonance (LSPR) band centered at 394 nm (Fig. 1a), which is characteristic of silver nanoparticles. The appearance of this plasmonic band indicates the reduction of  $\text{Ag}^+$  ions to metallic silver and the formation of nanoscale colloidal particles. Furthermore, the narrow and symmetric absorption profile suggests a relatively uniform particle size distribution with minimal aggregation, indicating good colloidal stability. In addition, the zeta potential of DTPA-AgNPs was further assessed (Fig. S1). The nanoparticles exhibited a zeta potential of approximately – 15.7 mV, indicating the presence of negatively charged species on the nanoparticle surface, arising from deprotonated

carboxylate groups of DTPA. Although this value indicates moderate electrostatic stabilization, the absence of extensive aggregation in the TEM images suggests that steric stabilization from adsorbed DTPA molecules also helps maintain nanoparticle dispersion. On the other hand, in the presence of creatinine, a gradual decrease in the LSPR band at 397 nm accompanied by the appearance of a new absorption band at 648 nm was observed, indicating nanoparticle aggregation induced by creatinine (Fig. 1a). As shown in the inset in Fig. 1a, the DTPA-AgNP dispersion exhibited a distinct color change from yellow to brown, implying naked-eye creatinine detection. This spectral and color change arises from interparticle plasmon coupling as the nanoparticles transition from dispersed to an aggregated state.

The crystalline structure of the synthesized nanoparticles was confirmed by X-ray diffraction (XRD) analysis (Fig. 1c). Diffraction peaks observed at  $2\theta$  values of approximately  $38.1^\circ$ ,  $44.3^\circ$ ,  $64.5^\circ$ , and  $77.4^\circ$  were indexed to the (111), (200), (220), and (311) planes of face-centered cubic (fcc) silver, respectively. The predominance of the (111) reflection indicates preferential crystal growth along this orientation. Furthermore, the sharp diffraction peaks demonstrate the high crystallinity and phase purity of the AgNPs, consistent with the faceted morphology observed by TEM. Such crystalline characteristics are advantageous for preserving the plasmonic properties required for sensitive colorimetric detection.

The XPS analysis was performed to identify the elemental composition of AgNPs. As shown in Fig. 1c and Fig. S3a, very strong intensity of Ag  $3d_{5/2}$  and Ag  $3d_{3/2}$  were observed at 367.83 eV and 374.03 eV, respectively. This binding energy indicates the existence of metallic Ag ( $\text{Ag}^0$ ), which confirms the silver nanoparticle formation [30]. Additionally, the presence of the C 1s, N 1s, and O 1s spectra is associated with the DTPA capping layer. Based on the high-resolution C 1s and O 1s XPS spectra shown in Fig. S2b and S2c, peaks appeared at 287.65 eV and 530.78 eV attributed to the carboxylate group of DTPA coordinated to the AgNP surface [31]. Furthermore, the N 1s exhibited a broad nitrogen contribution at 399.60 eV, which can be attributed primarily to amine-type nitrogen in the DTPA ligand [32]. The coexistence of these elements supports the successful incorporation of DTPA onto the AgNP surface.

FTIR spectroscopy was employed to verify the surface functionalization of AgNPs by DTPA (Fig. 1d). The FTIR spectrum of free DTPA exhibited characteristic absorption bands at  $1722\text{ cm}^{-1}$  and  $1573/1378\text{ cm}^{-1}$ , corresponding to carbonyl and carboxylate vibrations, respectively. Following nanoparticle synthesis, the carbonyl band at  $1722\text{ cm}^{-1}$  disappeared completely, while the carboxylate bands shifted to approximately  $1582$  and  $1396\text{ cm}^{-1}$ . Additionally, a reduction in the intensity of bands within the  $1000\text{--}1300\text{ cm}^{-1}$  region was observed. These spectral modifications indicate that the chemical environment of DTPA was altered upon nanoparticle formation. The disappearance of the carbonyl vibration together with the shifts in carboxylate-related bands suggests the involvement of carboxylate groups in the interaction with the AgNP surface, confirming the adsorption of DTPA onto the nanoparticles and its role as a capping ligand. This interpretation is further supported by the DFT-optimized structure, which indicates that DTPA coordinates to the Ag surface through its oxygen-containing carboxylate groups. Upon exposure to creatinine, a new peak appeared at  $1326\text{ cm}^{-1}$  that may be ascribed to the C–N bond of creatinine. The carboxylate vibration band of DTPA also red-shifted from  $1582$  to  $1573\text{ cm}^{-1}$ , indicating a change in the chemical environment of the carboxylate groups that is consistent with the formation of intermolecular hydrogen-bonding interactions [33]. This observation is in agreement with the subsequent QAIM and NCI analyses, which identified  $\text{O}\cdots\text{H}$  hydrogen bonds and additional  $\text{O}\cdots\text{N}$  contacts between creatinine and the DTPA-functionalized AgNP surface.

The morphology and size distribution of the synthesized nanoparticles were examined by transmission electron microscopy (TEM) (Fig. 1e). The nanoparticles exhibited predominantly quasi-spherical morphologies with good dispersion and minimal aggregation. Statistical analysis revealed an average particle diameter of  $24.70 \pm 0.30\text{ nm}$ . The presence of well-defined particle boundaries further indicates controlled nanoparticle growth during the reduction process. Creatinine's ability to induce AgNPs aggregation was also well presented in the TEM image (Fig. 1e). After mixing with creatinine, the stabilized AgNPs were dragged closer and formed an aggregate. To evaluate the particle size before and after creatinine addition, DLS measurement was performed. DTPA-AgNP size in the colloidal state yielded an average hydrodynamic diameter of approximately  $87.2\text{ nm}$  (PDI = 0.23) (Fig. S3). The substantially larger size compared with TEM measurements is attributed to the hydration layer and surface-bound DTPA molecules surrounding the nanoparticle core, providing additional evidence of successful surface functionalization. Upon creatinine addition, a notable shift to  $215.2\text{ nm}$  (PDI = 0.29) was observed (Fig. S2). This observation confirms the DTPA-AgNP aggregation when introduced to creatinine.

## 3.2. Interaction Mechanism between DTPA-AgNPs and Creatinine

To understand the principle of creatinine-induced AgNPs aggregation, the nature of surface charge interaction between DTPA and creatinine molecules was explored. As depicted in Scheme 1, the initial state of AgNPs before creatinine exposure was electrostatically stabilized by the carboxylic group, which strongly bound to the AgNPs surface. The negatively charged AgNPs surface leads to electrostatic repulsion between the molecules, which prevents nanoparticle aggregation [18]. At alkaline pH, creatinine undergoes an equilibrium tautomerization of amino and amine forms [34]. This transformation triggered the exposed remaining DTPA carboxylic acid groups to interact with creatinine via hydrogen bond formation [35]. As a result, the number of AgNPs surface negative charges was reduced and repulsion between the molecules, inducing aggregation.

## 3.3 Optimization of Experimental Variables by Response Surface Methodology

The effects of pH (Fig. 2a), AgNP concentration (Fig. 2b), and detection time (Fig. 2c) on the absorbance ratio ( $A_{648}/A_{397}$ ) were optimized using a Box–Behnken Design (BBD) coupled with Response Surface Methodology (RSM). The experimental data were fitted to a second-order polynomial model and evaluated by analysis of variance (ANOVA). The quadratic model was significant ( $F = 157.32$ ,  $p < 0.0001$ ), whereas the lack-of-fit was not ( $F = 3.51$ ,  $p = 0.2296$ ), indicating good agreement between the experimental and predicted responses. The model exhibited excellent fit with  $R^2$ , adjusted  $R^2$ , and predicted  $R^2$  values of 0.9965, 0.9901, and 0.9514, respectively. Furthermore, an Adequate Precision value of 39.54 indicated a satisfactory signal-to-noise ratio and confirmed the suitability of the model for optimization.

Among the investigated factors, AgNP concentration exerted the greatest influence on the response ( $F = 564.02$ ), followed by pH ( $F = 405.74$ ) and detection time ( $F = 380.71$ ). All linear terms were highly significant ( $p < 0.0001$ ). Significant AB and AC interactions ( $p < 0.05$ ) revealed that the effects of AgNP concentration and detection time were dependent on pH, whereas the BC interaction was not significant ( $p = 0.5828$ ). The significance of the  $A^2$  and  $C^2$  terms further indicated curvature in the response surface and the existence of optimum operating conditions. The relationship between the absorbance ratio and the coded variables was described by the following quadratic polynomial equation:

$$y = 0.3564 + 0.1218A + 0.1436B + 0.1180C + 0.0288AB + 0.0336AC + 0.0050BC - 0.0376A^2 + 0.0135B^2 - 0.0383C^2 \quad (2)$$

Numerical optimization predicted the maximum absorbance ratio at pH 11.81, an AgNP concentration of 141.31  $\mu\text{M}$ , and a detection time of 4.51 min. Under these conditions, the predicted response was 0.6545 with a desirability value of 1.000, indicating complete fulfilment of the optimization criteria.

## 3.4 Validation of Analytical Performance

The analytical sensitivity of the developed DTPA-AgNPs colorimetric sensor toward creatinine was evaluated using UV–Vis spectrophotometry under optimum conditions. As shown in Fig. 3a, the sensor exhibited a linear response over the concentration range of 5–90  $\mu\text{M}$ . The linear regression curve was obtained from the relationship between creatinine concentration and AgNPs absorbance ratio  $A_{648}/A_{397}$  (Fig. 3b). Additionally, the calibration curve followed the equation  $A_{648}/A_{397} = 0.0192 + 0.00625[C]$ . The excellent linearity observed ( $R^2 = 0.99283$ ,  $r = 0.99641$ ) indicates a strong correlation between creatinine concentration and the sensor's optical response. From the linear regression, the DTPA-AgNP detection limit (LOD) was calculated using  $\text{LOD} = 3\sigma/\text{slope}$ , where  $\sigma$  is the internal standard deviation, yielding 1.28  $\mu\text{M}$ .

Colorimetric detection relies on distinct changes in colour intensity, which are sensitive to the analyte concentration [36]. Therefore, RGB profile analysis of DTPA-AgNPs following creatinine addition was conducted as a complementary technique to spectroscopic analysis. In general, the colour analysis is performed by using digital imaging methods based on the three primary colour components: red, green, and blue (RGB). Initially, the DTPA-AgNPs solution was mixed with various concentrations of creatinine and then captured by a smartphone camera, followed by intensity determination using a color recognition app (Fig. 3c). As shown in Fig. 3d, the red and green channels exhibited stronger linear correlations with log-transformed creatinine concentration than the blue channel. The green channel exhibits higher correlation coefficients ( $R^2 = 0.99387$ ) among the three

RGB channels therefore selected for quantitative analysis, yielding an LOD of 0.19  $\mu\text{M}$ . Increasing creatinine concentration produced concentration-dependent changes in the RGB intensities, with the red and green intensities decreasing while the blue intensity increased. To further characterize the overall color change, the Euclidean color distance ( $\Delta d$ ) between the RGB values before and after creatinine exposure was calculated using Eq. 1. The resulting  $\Delta d$  values increased systematically with creatinine concentration and showed a strong linear relationship with log-transformed creatinine concentration (Fig. 3e), providing a complementary descriptor of the overall colorimetric response.

### 3.5 DTPA-AgNP probe selectivity toward creatinine

The selectivity of the DTPA-AgNP system toward creatinine was evaluated in the presence of various potential interfering substances, including metal ions ( $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ) and bioorganic compounds such as urea, glucose, cysteine, and ascorbic acid (Fig. 4a and 4b). In contrast, the addition of creatinine resulted in a distinct spectral transformation, characterized by the emergence of a new absorption band at 648 nm. The observed selectivity can be attributed to the specific interaction between creatinine and the DTPA-functionalized nanoparticle surface. The presence of multiple functional groups in both species enables favourable intermolecular interactions, which can promote nanoparticle bridging and subsequent aggregation. In contrast, other tested analytes either lack suitable interaction sites or are unable to induce sufficient interparticle coupling to generate a measurable optical response.

### 3.6 Application of creatinine assay in real human urine samples

As creatinine is primarily excreted in urine and serves as an important biomarker of kidney-related diseases, human urine is the most relevant biological sample for evaluating the clinical applicability of the proposed colorimetric sensor. Thus, we assessed the accuracy of the developed method using the spike recovery approach in urine samples (Table 2 and Fig S4). For the UV-Vis method, the percentage recoveries ranged from 89.7 to 109.2%, whereas the digital image method yielded recoveries between 103.3 and 108.6%. These recovery values indicate that the proposed sensor can accurately quantify creatinine in urine samples with negligible matrix interference. The obtained results fall within the acceptable recovery range according to the Association of Official Analytical Chemists (2020), which stated that the good recovery range lies between 85 and 115% [37], confirming the accuracy and reliability of the developed method. The precision of the proposed sensing platform was assessed through repeated measurements and expressed as the relative standard deviation (%RSD). The %RSD values were calculated from the ratio of the standard deviation to the corresponding analytical response. Both methods demonstrated %RSD values below 10%, indicating excellent repeatability and analytical reliability [38].

Table 2  
Spike-recovery experiment using creatinine addition technique in human urine samples with UV-Vis and digital image method analysis.

| Sample (n = 3) | UV-Vis method           |                         |         |              | Digital image method    |                         |         |              |
|----------------|-------------------------|-------------------------|---------|--------------|-------------------------|-------------------------|---------|--------------|
|                | Added ( $\mu\text{M}$ ) | Found ( $\mu\text{M}$ ) | RSD (%) | Recovery (%) | Added ( $\mu\text{M}$ ) | Found ( $\mu\text{M}$ ) | RSD (%) | Recovery (%) |
| Human urine    | 0                       | 0.88                    | 5.75    | -            | 0                       | 1.38                    | 2.21    | -            |
|                | 10                      | 11.84                   | 1.82    | 109.2        | 10                      | 11.71                   | 1.98    | 103.3        |
|                | 30                      | 32.71                   | 4.13    | 106.1        | 30                      | 33.97                   | 3.99    | 108.6        |
|                | 50                      | 45.75                   | 8.76    | 89.7         | 50                      | 53.10                   | 3.67    | 103.4        |

### 3.7 Comparison study of proposed method

A comparison of the present work with previously reported methods and materials is listed in Table 3. From the presented list, our developed method exhibited more sensitive and rapid creatinine detection. Moreover, the digital image method offers excellent practicality through direct image capture with simple tools, enabling real-time clinical diagnosis. Other previous methods, for example, DNA aptamer-AuNP conjugates and Gold Nanoparticle-Peptide probes, used high-cost materials, complicated synthesis, and longer incubation times for creatinine detection [9, 39], which limit reproducibility.

Table 3  
Comparison table with other reported materials for creatinine detection.

| Method / Nanoparticle Type                                  | Dynamic Range   | LOD           | Detection Time | Reference    |
|-------------------------------------------------------------|-----------------|---------------|----------------|--------------|
| DNA aptamer-AuNP conjugates                                 | 2–20 mM         | 0.87 mM       | 5 min          | [39]         |
| AgNP/polyvinyl alcohol (PVA)/Zeolitic Imidazolate Framework | 0–150 $\mu$ M   | 68.5 $\mu$ M  | 3.17 min       | [40]         |
| Gold Nanoparticle-Peptide probes                            | 0–17,68 $\mu$ M | 0.220 $\mu$ M | 16 min         | [9]          |
| Citrate-Silver Nanoparticle                                 | 2–2,5 mM        | 55 nM         | 30 min         | [41]         |
| CdTe@MOFs                                                   | 0–300 $\mu$ M   | 1.78 $\mu$ M  | 2 min          | [42]         |
| DTPA-AgNP(UV-method)                                        | 0–90 $\mu$ M    | 1.28 $\mu$ M  | 4.51 min       | Present work |
| DTPA-AgNP(Digital image method)                             |                 | 0.19 $\mu$ M  |                |              |

### 3.8 Atomic-Level Insights into the Creatinine Recognition Mechanism

Density functional theory (DFT) calculations were performed to elucidate the molecular origin of creatinine recognition by DTPA-modified silver nanoparticles (DTPA-AgNPs). The optimized adsorption geometry indicates that DTPA strongly coordinates to the Ag cluster through its oxygen-containing carboxylate groups, producing a stable ligand shell while leaving accessible adsorption sites for creatinine. Similar coordination behavior has been reported for multidentate carboxylate ligands on noble-metal nanoparticles, where ligand coordination simultaneously enhances colloidal stability and provides active recognition sites for analyte adsorption [43, 44].

The calculated thermodynamic parameters (Table 4) demonstrate that DTPA functionalization markedly enhances the adsorption affinity of silver nanoparticles toward creatinine. The adsorption energy becomes more negative, decreasing from –160.12 kcal mol<sup>–1</sup> for DTPA-AgNP to –175.81 kcal mol<sup>–1</sup> after creatinine adsorption, while the Gibbs free energy remains highly negative ( $\Delta G = -348.93$  kcal mol<sup>–1</sup>), confirming that complex formation is spontaneous and thermodynamically favorable. In contrast, creatinine adsorption on bare AgNPs is considerably weaker ( $E_{\text{ads}} = -4.58$  kcal mol<sup>–1</sup>), highlighting the crucial role of DTPA in promoting molecular recognition. Similar trends have been reported for ligand-functionalized metal nanostructures, where surface ligands substantially increase adsorption energies and improve analyte selectivity through cooperative hydrogen bonding and electrostatic interactions [45, 46].

Table 4  
Calculated physicochemical, thermodynamic, and quantum chemical descriptors of DTPA-AgNP, AgNP/creatinine, and DTPA-AgNP/creatinine complexes obtained at the DFT/M06-2X/6-311G(d,p)/LanL2DZ level.

| Parameters                                                    | DTPA-AgNP | Cre/AgNP | Cre/DTPA-AgNP |
|---------------------------------------------------------------|-----------|----------|---------------|
| HOMO, $E_{\text{HOMO}}$ (eV)                                  | 5.67      | -4.62    | 5.20          |
| LUMO, $E_{\text{LUMO}}$ (eV)                                  | 7.16      | -2.11    | 6.94          |
| Energy gap, $E_g$ (eV)                                        | 1.49      | 2.51     | 1.74          |
| Work function, $\Phi$ (eV)                                    | -6.41     | 3.37     | -6.07         |
| Chemical potential, $\mu$ (eV)                                | 6.41      | -3.37    | 6.07          |
| Hardness, $\eta$ (eV)                                         | 0.74      | 1.25     | 0.87          |
| Electrophilicity, $\omega$ (eV)                               | 27.63     | 4.53     | 21.17         |
| Softness, $S$ (eV)                                            | 0.67      | 0.40     | 0.57          |
| Adsorption energy, $E_{\text{ads}}$ (kcal mol <sup>-1</sup> ) | -160.12   | -4.58    | -175.81       |
| Entropy, $\Delta S$ (cal mol <sup>-1</sup> K <sup>-1</sup> )  | -220.18   | -190.49  | -382.99       |
| Enthalpy, $\Delta H$ (kcal mol <sup>-1</sup> )                | -179.72   | -23.86   | -463.12       |
| Gibbs free energy, $\Delta G$ (kcal mol <sup>-1</sup> )       | -114.07   | 32.93    | -348.93       |

Frontier molecular orbital (FMO) analysis further reveals that creatinine adsorption modifies the electronic structure of DTPA-AgNPs (Fig. 5a). The HOMO–LUMO energy gap increases from 1.49 eV for DTPA-AgNP to 1.74 eV after creatinine adsorption, accompanied by an increase in chemical hardness (0.74 to 0.87 eV) and a decrease in softness (0.67 to 0.57 eV) (Table 4). According to conceptual density functional theory, an increase in HOMO–LUMO energy gap and hardness generally reflects enhanced electronic stability and reduced chemical reactivity of the adsorption complex [44, 47]. These findings indicate that creatinine adsorption stabilizes the electronic structure of the DTPA-functionalized nanoparticle.

The electrostatic potential (ESP) map (Fig. 5b) provides additional insight into the adsorption process. Electron-rich regions are predominantly localized around the carboxylate oxygen atoms of DTPA, whereas relatively electron-deficient regions are distributed over the silver cluster. This complementary electrostatic distribution creates favorable interaction sites for the amino and carbonyl groups of creatinine through electrostatic attraction and hydrogen bonding. Previous DFT investigations have shown that ESP mapping is an effective tool for identifying preferential adsorption sites and predicting molecular recognition on functionalized nanomaterial surfaces [48, 49].

The density of states (DOS) and charge density difference (CDD) analyses further clarify the electronic interactions governing adsorption (Fig. 5c). The DOS spectra exhibit redistribution of electronic states near the frontier orbital region after creatinine adsorption, indicating orbital hybridization between creatinine and the DTPA-functionalized nanoparticle. Concurrently, the CDD isosurface reveals electron accumulation around the DTPA ligand and creatinine, accompanied by electron depletion on adjacent silver atoms, confirming intermolecular charge transfer across the adsorption interface. Similar electronic redistribution has been reported in DFT studies of ligand-functionalized noble-metal nanoparticles, where charge transfer between the ligand, analyte, and metal surface plays a key role in modulating localized surface plasmon resonance and sensing performance [50].

Taken together, the thermodynamic and electronic structure analyses consistently demonstrate that DTPA functionalization substantially enhances the affinity of AgNPs toward creatinine by providing favorable adsorption sites and facilitating intermolecular charge transfer. The strong adsorption and accompanying electron redistribution modify the surface electronic environment of DTPA-AgNPs, promoting intermolecular interactions between neighboring nanoparticles. This mechanism provides an atomistic explanation for the experimentally observed aggregation-induced colorimetric response and supports the high sensitivity of the proposed dual-mode DTPA-AgNP sensing application.

### 3.9 QTAIM and NCI Analyses of the Creatinine Adsorption Mechanism

To obtain a deeper understanding of the intermolecular interactions governing creatinine adsorption, the optimized DTPA-AgNP and Cre/DTPA-AgNP complexes were analyzed using QTAIM and NCI methods. The optimized geometries, reduced density gradient (RDG) scatter plots, and NCI isosurfaces are presented in Fig. 6a and Fig. 6b, while the corresponding topological parameters at the bond critical points (BCPs) are summarized in Table S1. QTAIM analysis reveals that the DTPA ligand is anchored to the silver cluster primarily through multiple Ag $\cdots$ O coordination interactions, with electron density values ( $\rho_{bcp}$ ) ranging from 0.0162 to 0.0480 a.u. in the DTPA-AgNP complex. Upon creatinine adsorption, the strongest Ag $\cdots$ O interaction increases to  $\rho_{bcp} = 0.0550$  a.u., accompanied by a more negative total energy density ( $H_{bcp} = -0.0028$  a.u.), indicating reinforcement of the Ag–O coordination environment. According to the QTAIM criteria established by Bader and further refined by Espinosa et al., the positive Laplacian values ( $\nabla^2_{bcp} > 0$ ) together with the  $-G/V$  ratios ranging from 0.965 to 1.227 suggest that these Ag $\cdots$ O contacts are predominantly closed-shell interactions with varying degrees of covalent contribution, where values below unity indicate partial covalent character and values exceeding unity correspond mainly to electrostatic interactions. Similar bonding characteristics have recently been reported for functionalized silver nanostructures, where metal–oxygen coordination plays a crucial role in stabilizing adsorbed biomolecules and enhancing sensing performance [27, 51].

Besides strengthening the Ag $\cdots$ O coordination network, creatinine adsorption introduces additional intermolecular contacts that are absent in the isolated DTPA-AgNP system, including O $\cdots$ H hydrogen bonds and an O $\cdots$ N interaction. Among these, the strongest O $\cdots$ H interaction exhibits  $\rho_{bcp} = 0.0421$  a.u.,  $H_{bcp} = -0.0025$  a.u., and  $-G_{bcp}/V_{bcp} = 0.942$ , indicating a relatively strong hydrogen bond with partial covalent character, whereas the remaining O $\cdots$ H and O $\cdots$ N interactions possess lower electron densities (0.0103–0.0130 a.u.) and  $-G_{bcp}/V_{bcp} > 1$ , characteristic of weaker electrostatic interactions. The coexistence of these interactions demonstrates that creatinine adsorption is governed by a cooperative interaction network consisting of metal coordination, hydrogen bonding, and weak electrostatic contacts rather than a single dominant interaction. Such synergistic binding has also been observed in recent computational and spectroscopic investigations of creatinine adsorption on plasmonic nanomaterials, where hydrogen-bond-assisted molecular recognition significantly improves adsorption stability and sensing efficiency [52, 53].

The NCI analysis further corroborates the QTAIM results. As shown in Fig. 6, the RDG scatter plots exhibit pronounced spikes within the negative  $\text{sign}(\lambda_2)\rho$  region, confirming the presence of attractive intermolecular interactions. Compared with the isolated DTPA-AgNP complex, the DTPA-AgNP/creatinine complex displays more extensive green NCI isosurfaces distributed over the adsorption interface, indicating that creatinine adsorption is stabilized by multiple cooperative van der Waals interactions. Localized blue isosurfaces appear around the Ag $\cdots$ O coordination sites and hydrogen-bonding regions, corresponding to stronger attractive interactions identified by the QTAIM analysis, whereas only limited red isosurfaces associated with steric repulsion are observed. This distribution of NCI isosurfaces is characteristic of stable adsorption complexes and agrees well with the reduced density gradient formalism proposed by Johnson et al. and the NCIPLOT methodology developed by Contreras-García et al., which have become standard approaches for visualizing weak intermolecular interactions in adsorption systems [28, 29].

These complementary QTAIM and NCI analyses demonstrate that DTPA functionalization establishes a cooperative interaction network involving Ag $\cdots$ O coordination, hydrogen bonding, and dispersion interactions, thereby substantially enhancing the adsorption affinity of creatinine toward the nanoparticle surface (Table S1). The strengthened coordination environment, together with the observed electron-density redistribution, provides an atomistic explanation for the experimentally observed aggregation of DTPA-functionalized AgNPs and the resulting colorimetric response. This adsorption mechanism is consistent with recent studies reporting that ligand-assisted coordination and hydrogen-bonding networks govern analyte-induced

aggregation and optical responses in silver nanoparticle-based sensing platforms, thereby supporting the proposed molecular recognition mechanism of the present dual-mode creatinine sensor [51, 53].

## 4. Conclusion

A dual-mode colorimetric sensing platform based on DTPA-functionalized silver nanoparticles (DTPA-AgNPs) was successfully developed for the sensitive, rapid, and selective detection of creatinine. DTPA acted as a multidentate capping ligand that stabilized the AgNPs through negatively charged carboxylate groups while providing accessible surface functionalities for creatinine recognition. RSM-BBD method successfully optimized the sensing conditions, yielding optimum parameters of pH 11.81, AgNP concentration of 141.31  $\mu\text{M}$ , and a detection time of 4.51 min. Under these conditions, the DTPA-AgNP sensor exhibited a linear response toward creatinine using UV–Vis spectroscopy, yielding an LOD of 1.28  $\mu\text{M}$ . In addition, smartphone-assisted RGB image analysis provided a complementary quantitative approach, with the green channel showing the highest analytical sensitivity and exhibiting a lower LOD value of 0.19  $\mu\text{M}$ . The sensor also demonstrated excellent selectivity against common inorganic ions and biomolecules. Computational analyses further revealed favorable interactions between creatinine and DTPA-AgNP, providing molecular-level support for the experimentally observed aggregation and colorimetric response. These findings demonstrate the potential of DTPA-AgNPs as a simple, sensitive, and portable platform for creatinine determination.

## Declarations

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### Author contributions

M.A.R. conceived the research idea, designed the methodology, conducted the investigation, curated the data, provided resources, and contributed to the preparation of the original manuscript. S.A.P.S. contributed to data analysis and validation and resources and critically revised the manuscript. L.A.M. contributed to data curation, formal analysis, investigation, and software, and contributed to the preparation of the original manuscript. M.R.B. contributed to data curation and critically revised the manuscript. N.F.S. contributed to investigation, data curation, formal analysis, and visualization, and contributed substantially to the preparation of the original manuscript and its critical revision. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for the work.

### Data availability

The data presented in this study are available in the article/Supporting Information.

**Competing Interests** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

1. Sheikh MS, Kashani KB (2025) Beyond creatinine: New methods to measure renal function? *Eur J Intern Med* 134:17–24. <https://doi.org/https://doi.org/10.1016/j.ejim.2025.01.015>

2. Okoye NC, Master SR, Hoofnagle AN, Miller WG (2026) Milestones in Kidney Function Testing: Reflecting on the Journey Toward Serum Creatinine Measurement Standardization and Its Impact on Chronic Kidney Disease Diagnosis and Management. *Arch Pathol Lab Med* 150:118–121. <https://doi.org/10.5858/arpa.2025-0431-RA>
3. Ávila M, Mora Sánchez MG, Bernal Amador AS, Paniagua R (2025) The Metabolism of Creatinine and Its Usefulness to Evaluate Kidney Function and Body Composition in Clinical Practice. *Biomolecules* 15:41
4. Mussap M (2023) In: Ciaccio M (ed) *Kidney BT - Clinical and Laboratory Medicine Textbook*. Springer International Publishing, Cham, pp 237–252
5. Jaffe M (1886) Ueber den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins. 10:391–400. <https://doi.org/doi:10.1515/bchm1.1886.10.5.391>
6. Narimani R, Esmaeili M, Rasta SH, Khosroshahi HT, Mobed A (2021) Trend in creatinine determining methods: Conventional methods to molecular-based methods. *Anal Sci Adv* 2:308–325. <https://doi.org/https://doi.org/10.1002/ansa.202000074>
7. Tkaczyk A, Jedziniak P (2020) Dilute-and-Shoot HPLC-UV Method for Determination of Urinary Creatinine as a Normalization Tool in Mycotoxin Biomonitoring in Pigs. *Molecules* 25:2445
8. Costa ACO, da Costa JL, Tonin FG, Tavares MFM, Micke GA (2007) Development of a fast capillary electrophoresis method for determination of creatinine in urine samples. *J Chromatogr A* 1171:140–143. <https://doi.org/https://doi.org/10.1016/j.chroma.2007.09.029>
9. Feng S, Shi R, Xu P, Bhamore JR, Bal J, Baek SH, Park CY, Park JP, Park TJ (2020) Colorimetric detection of creatinine using its specific binding peptides and gold nanoparticles. *New J Chem* 44:15828–15835. <https://doi.org/10.1039/D0NJ03860A>
10. Zhang K, Sun H, Wei L, Hu R, Liu H, Lai Y, Li X (2025) Robust and sensitive colorimetric detection of glutathione with double-triggering MOF-Fe(DTNB). *Spectrochim Acta Part Mol Biomol Spectrosc* 328:125439. <https://doi.org/https://doi.org/10.1016/j.saa.2024.125439>
11. Lu Y, Shen N, Xi Y, Zhu T, Peng H, Zhong L, Li F (2023) Bioenzyme-free colorimetric assay for creatinine determination based on Mn3O4 nanoparticles catalyzed oxidation of 3,3',5,5'-tetramethylbenzidine. *Microchim Acta* 191:44. <https://doi.org/10.1007/s00604-023-06129-8>
12. Zhu J, Pan J, Li Y, Yang J, Ye B (2022) Enzyme-nanozyme cascade colorimetric sensor platform: a sensitive method for detecting human serum creatinine. *Anal Bioanal Chem* 414:6271–6280. <https://doi.org/10.1007/s00216-022-04199-w>
13. Alzoubi FY, Ahmad AA, Aljarrah IA, Migdadi AB, Al-Bataineh QM (2023) Localize surface plasmon resonance of silver nanoparticles using Mie theory. *J Mater Sci Mater Electron* 34:2128. <https://doi.org/10.1007/s10854-023-11304-x>
14. Mayer KM, Hafner JH (2011) Localized Surface Plasmon Resonance Sensors. *Chem Rev* 111:3828–3857. <https://doi.org/10.1021/cr100313v>
15. Lv X, Shi T, Bai X, Guan Z, Wang H, Jiang R, Zhou L, Chen H (2025) A Simple and Efficient Colorimetric Detection of Creatinine Based on Citrate-Stabilized Gold Nanoparticles. *Plasmonics* 20:2753–2763. <https://doi.org/10.1007/s11468-024-02510-2>
16. Saputra E (2024) A colorimetric detection of creatinine based-on EDTA capped-gold nanoparticles (EDTA-AuNPs): Digital Image Colorimetry. *Sens Int* 5:100286. <https://doi.org/https://doi.org/10.1016/j.sintl.2024.100286>
17. Mohammadi S, Khayatian G (2015) Highly selective and sensitive photometric creatinine assay using silver nanoparticles. *Microchim Acta* 182:1379–1386. <https://doi.org/10.1007/s00604-015-1460-5>
18. Bhatt R, Bhatt R, P. P (2018) DTPA capped gold and silver nanofluids-facile synthesis and their application as chromium sensors. *Sens Actuators B Chem* 258:602–611. <https://doi.org/https://doi.org/10.1016/j.snb.2017.11.154>
19. Parhizgar Keradeh M, Tabatabaei-Nezhad SA (2023) Comprehensive analysis of the effect of reservoir key parameters on the efficacy of DTPA chelating agent in minimizing interfacial tension and enhanced oil recovery. *Results Eng* 19:101316. <https://doi.org/https://doi.org/10.1016/j.rineng.2023.101316>
20. Janah IM, Roto R, Konishi K, Siswanta D (2022) EDTA-capped silver nanoparticles as a probe for highly sensitive and selective colorimetric sensing of creatinine and optimization using response surface methodology-Box Behnken Design. *Talanta Open* 6:100170. <https://doi.org/https://doi.org/10.1016/j.talo.2022.100170>
21. Nubatonis YK, Roto R, Siswanta D, Keikimanova M, Hosseini-Bandegharaei A (2025) Smartphone colorimetry for rapid environmental monitoring: Detecting Ni<sup>2+</sup> + using EDTA and mercapto succinic acid functionalized silver nanoparticles. *J Mol*

Liq 426:127351. <https://doi.org/https://doi.org/10.1016/j.molliq.2025.127351>

22. Frisch MJ, Trucks G, Schlegel HB, Scuseria GE, Robb MA, Cheeseman J, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg J, Hada M, Fox D (2009) Gaussian 09 Revision A.1. Gaussian Inc
23. Zhao Y, Truhlar DG, Zhao Y, Truhlar · DG (2007) The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functio.... *Theor Chem Acc* 2007 1201(120):215–241. <https://doi.org/10.1007/S00214-007-0310-X>
24. Hay PJ, Wadt WR (1985) Ab initio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals. *J Chem Phys* 82:299–310. <https://doi.org/10.1063/1.448975>
25. Hay PJ, Wadt WR (1985) Ab initio effective core potentials for molecular calculations. Potentials for the transition metal atoms Sc to Hg. *J Chem Phys* 82:270–283. <https://doi.org/10.1063/1.448799>
26. Boys SF, Bernardi F (1970) The calculation of small molecular interactions by the differences of separate total energies. Some procedures with reduced errors. *Mol Phys* 19:553–566. <https://doi.org/10.1080/00268977000101561>
27. Espinosa E, Molins E, Lecomte C (1998) Hydrogen bond strengths revealed by topological analyses of experimentally observed electron densities. *Chem Phys Lett* 285:170–173. [https://doi.org/10.1016/S0009-2614\(98\)00036-0](https://doi.org/10.1016/S0009-2614(98)00036-0)
28. Johnson ER, Keinan S, Mori-Sánchez P, Contreras-García J, Cohen AJ, Yang W (2010) Revealing Noncovalent Interactions. *J Am Chem Soc* 132:6498–6506. <https://doi.org/10.1021/JA100936W>
29. Contreras-García J, Johnson ER, Keinan S, Chaudret R, Piquemal JP, Beratan DN, Yang W (2011) NCIPLOT: A Program for Plotting Noncovalent Interaction Regions. *J Chem Theory Comput* 7:625–632. <https://doi.org/10.1021/CT100641A>
30. Lee SJ, Guan Z, Xu H, Moskovits M (2007) Surface-Enhanced Raman Spectroscopy and Nanogeometry: The Plasmonic Origin of SERS. *J Phys Chem C* 111:17985–17988. <https://doi.org/10.1021/JP077422G>
31. Chiu NF, Tai MJ, Nurrohman DT, Lin TL, Wang YH, Chen CY (2021) Immunoassay-amplified responses using a functionalized mos2-based spr biosensor to detect papp-a2 in maternal serum samples to screen for fetal down's syndrome. *Int J Nanomed* 16:2715–2733. <https://doi.org/10.2147/IJN.S296406>
32. Siddiqi HM, Afzal A, Sajid S, Akhter Z (2013) Synthesis, characterization and thermal oxidative stability of rigid epoxy polymers cured from aromatic mono- and di-amines. *J Polym Res* 20. <https://doi.org/10.1007/S10965-012-0041-0>
33. Fornaro T, Burini D, Biczysko M, Barone V (2015) Hydrogen-Bonding Effects on Infrared Spectra from Anharmonic Computations: Uracil–Water Complexes and Uracil Dimers. *J Phys Chem A* 119:4224–4236. <https://doi.org/10.1021/acs.jpca.5b01561>
34. Alula MT, Karamchand L, Hendricks NR, Blackburn JM (2018) Citrate-capped silver nanoparticles as a probe for sensitive and selective colorimetric and spectrophotometric sensing of creatinine in human urine. *Anal Chim Acta* 1007:40–49. <https://doi.org/https://doi.org/10.1016/j.aca.2017.12.016>
35. Rahayu MA, Siboro SAP, Marlina LA, Suhendra NF, Yusnaidar Y, Wulandari Y, Malay R (2026) Mechanistic insight into creatinine detection via tartrate-stabilized silver nanoparticles: A combined experimental and DFT study. *Spectrochim Acta Part Mol Biomol Spectrosc* 357:127481. <https://doi.org/https://doi.org/10.1016/j.saa.2026.127481>
36. Lobregas MOS, Bantang JPO, Camacho DH (2019) Carrageenan-stabilized silver nanoparticle gel probe kit for colorimetric sensing of mercury (II) using digital image analysis. *Sens Bio-Sensing Res* 26:100303. <https://doi.org/https://doi.org/10.1016/j.sbsr.2019.100303>
37. Saputra E, Kase Z, Timur I, Frisky A, Akbar Wicaksono ND, Jatmiko W, Munawwaroh Z, Alimin, Pambudi F, Santosa S (2025) A novel method utilizing a portable digital image-based colorimeter detector and a glutathione-functionalized gold nanoparticles (GSH-AuNPs) sensor for the highly selective and sensitive detection of creatinine in urine sample. *Sens Int* 6:100327. <https://doi.org/10.1016/j.sintl.2025.100327>
38. Suhendra NF, Lee H (2025) Fluorescent polymeric thin films for selective and sensitive paraquat detection and removal from contaminated environmental media. *J Hazard Mater* 494:138513. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2025.138513>

39. Das C, Raveendran J, Bayry J, Rasheed PA (2024) Selective and naked eye colorimetric detection of creatinine through aptamer-based target-induced passivation of gold nanoparticles. *RSC Adv* 14:33784–33793. <https://doi.org/10.1039/D4RA06191H>
40. Azeman NH, Yusof K, Zafar Ali N, Aziz FHA, Othman MQ, Nazri NAA, Bakar MHA, Zain ARM, Jamil MSM, Bakar AAA (2026) Plasmonic AgNP/PVA/ZIF-8 Hybrid Framework for Enhanced Sensitivity and Selectivity in Creatinine Detection. *IEEE Sens J* 26:3625–3638. <https://doi.org/10.1109/JSEN.2025.3644319>
41. Nguyen T, Chang C-P, Yuan S-M (2017) A Wi-Fi Union Mechanism for Internet Advertising Reciprocal Platform in Microenterprises. *Sensors* 17:1617. <https://doi.org/10.3390/s17071617>
42. Guan J, Xiong Y, Wang M, Liu Q, Chen X (2024) A novel functionalized CdTe@MOFs based fluorometric and colorimetric biosensor for dual-readout assay of creatinine. *Sens Actuators B Chem* 399:134842. <https://doi.org/10.1016/j.snb.2023.134842>
43. Grimme S (2011) Density functional theory with London dispersion corrections. *Wiley Interdiscip Rev Comput Mol Sci* 1:211–228. <https://doi.org/10.1002/WCMS.30>;CTYPE:STRING:JOURNAL
44. Geerlings P, De Proft F, Langenaeker W (2003) Conceptual Density Functional Theory. *Chem Rev* 103:1793–1874. <https://doi.org/10.1021/CR990029P>
45. Liu W, Li Y, Wang Y, Zhao Y, Xu Y, Liu X (2022) DFT insights into the degradation mechanism of carbendazim by hydroxyl radicals in aqueous solution. *J Hazard Mater* 431:128577. <https://doi.org/10.1016/J.JHAZMAT.2022.128577>
46. Wang B, Zhu M, Liu M, Wang Y, Zhou Y, Ma J (2022) Design of novel dual functional ionic liquids and DFT study on their CO<sub>2</sub> absorption mechanism. *J Mol Liq* 366:120340. <https://doi.org/10.1016/J.MOLLIQ.2022.120340>
47. Parr RG, Yang W (1989) *Density Functional Theory of Atoms and Molecules*. Oxford University Press, Oxford. - References - Scientific Research Publishing. <https://www.scirp.org/reference/referencespapers?referenceid=2563317>. Accessed 1 Aug 2026
48. Politzer P, Murray JS (2002) The fundamental nature and role of the electrostatic potential in atoms and molecules. *Theor Chem Acc* 108:134–142. <https://doi.org/10.1007/S00214-002-0363-9>/METRICS
49. Murray JS, Politzer P (2011) The electrostatic potential: An overview. *Wiley Interdiscip Rev Comput Mol Sci* 1:153–163. <https://doi.org/10.1002/WCMS.19>;SUBPAGE:STRING:ABSTRACT;WEBSITE:WEBSITE:WIRES;WGROU:STRING:PUBLICATION
50. Zhou L, Zhang G, Xiu F, Xia S, Yu L (2020) The tesseract in two dimensional materials, a DFT approach. *RSC Adv* 10:8618–8627. <https://doi.org/10.1039/C9RA10696K>
51. Morad R (2025) Investigating adsorption of 5-fluorouracil and 6-mercaptopurine on icosahedral silver nanoparticles through DFT and TDDFT. *Sci Reports* 2025 15: 41839. <https://doi.org/10.1038/s41598-025-25773-z>
52. Atta S, Vo-Dinh T (2024) Improved solution-based SERS detection of creatinine by inducing hydrogen-bonding interaction for effective analyte capture. *Talanta* 278:126373. <https://doi.org/10.1016/J.TALANTA.2024.126373>
53. Huang J, Sokolikova M, Ruiz-Gonzalez A, Kong Y, Wang Y, Liu Y, Xu L, Wang M, Mattevi C, Davenport A, Lee TC, Li B (2024) Ultrasensitive colorimetric detection of creatinine via its dual binding affinity for silver nanoparticles and silver ions. *RSC Adv* 14:9114–9121. <https://doi.org/10.1039/D3RA08736K>

## Scheme 1

Scheme 1 is available in the Supplementary Files section.

## Figures

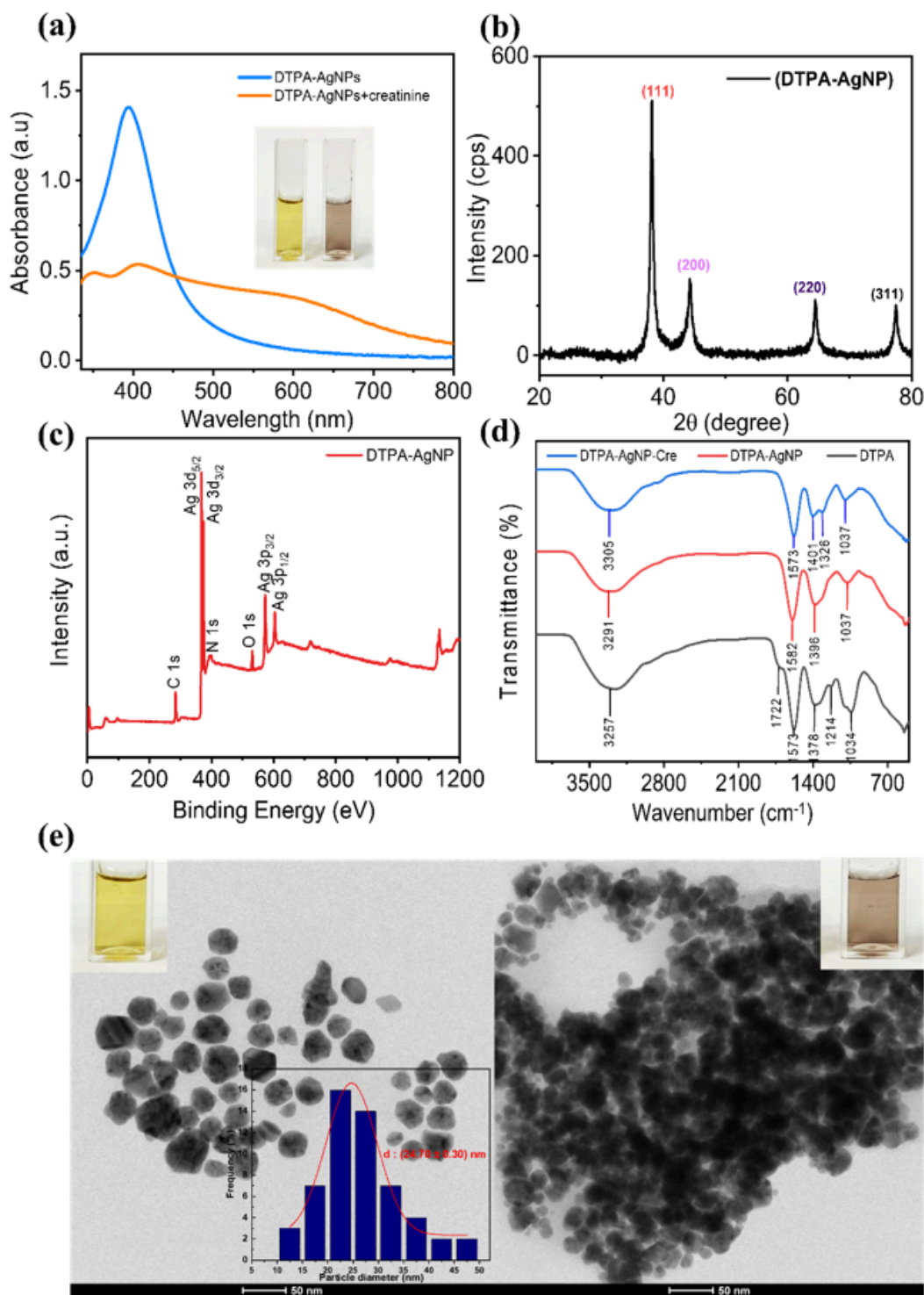


**Figure 1**

(a) UV-Visible spectral changes of DTPA-AgNPs (blue line) and DTPA-AgNPs after interaction with creatinine (orange line) at pH 11 and a creatinine concentration of 90  $\mu$ M. The inset shows the corresponding color change of the DTPA-AgNP colloids from yellow to grey upon creatinine addition, from which the UV-Vis spectra were recorded. (b) XRD pattern of DTPA-AgNP confirming DTPA-AgNP crystallinity. (c) XPS survey spectra of DTPA-AgNP showing the binding energy of each elemental composition. (d) Comparative FTIR spectra of pure DTPA, AgNP-DTPA, and AgNP-DTPA after interaction with creatinine. (e) TEM image of dispersed DTPA-capped silver nanoparticles before creatinine addition (left). Inset: particle size distribution

histogram of DTPA–AgNPs. TEM image illustrating the aggregation of DTPA–AgNPs upon interaction with creatinine under optimal conditions (right).

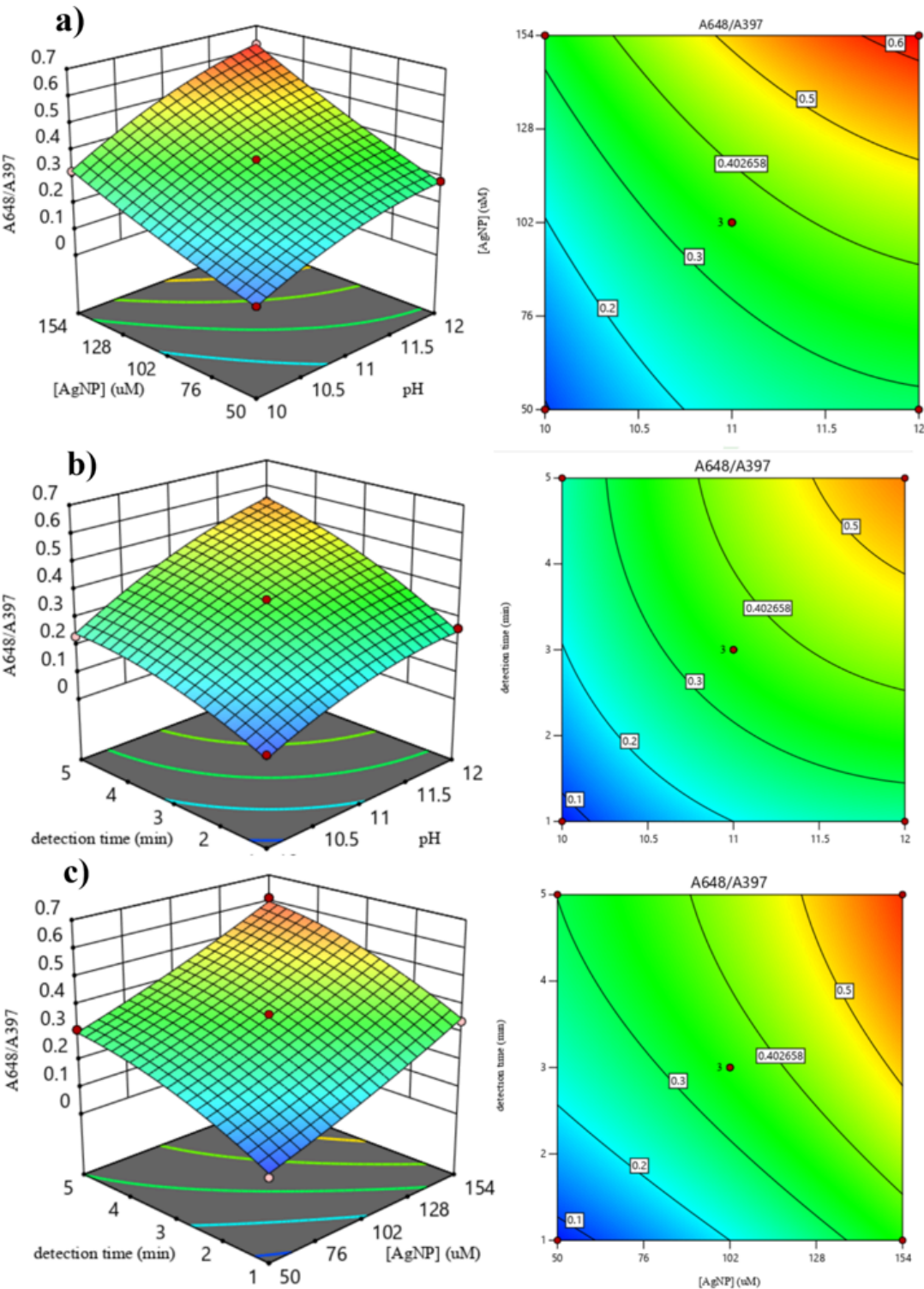


Figure 2

Response surface plots illustrating the interactive effects of (a) pH and DTPA concentration, (b) pH and AgNP concentration, and (c) DTPA concentration and AgNP concentration on the A648/A397 response of the DTPA-modified AgNPs-based creatinine sensor.

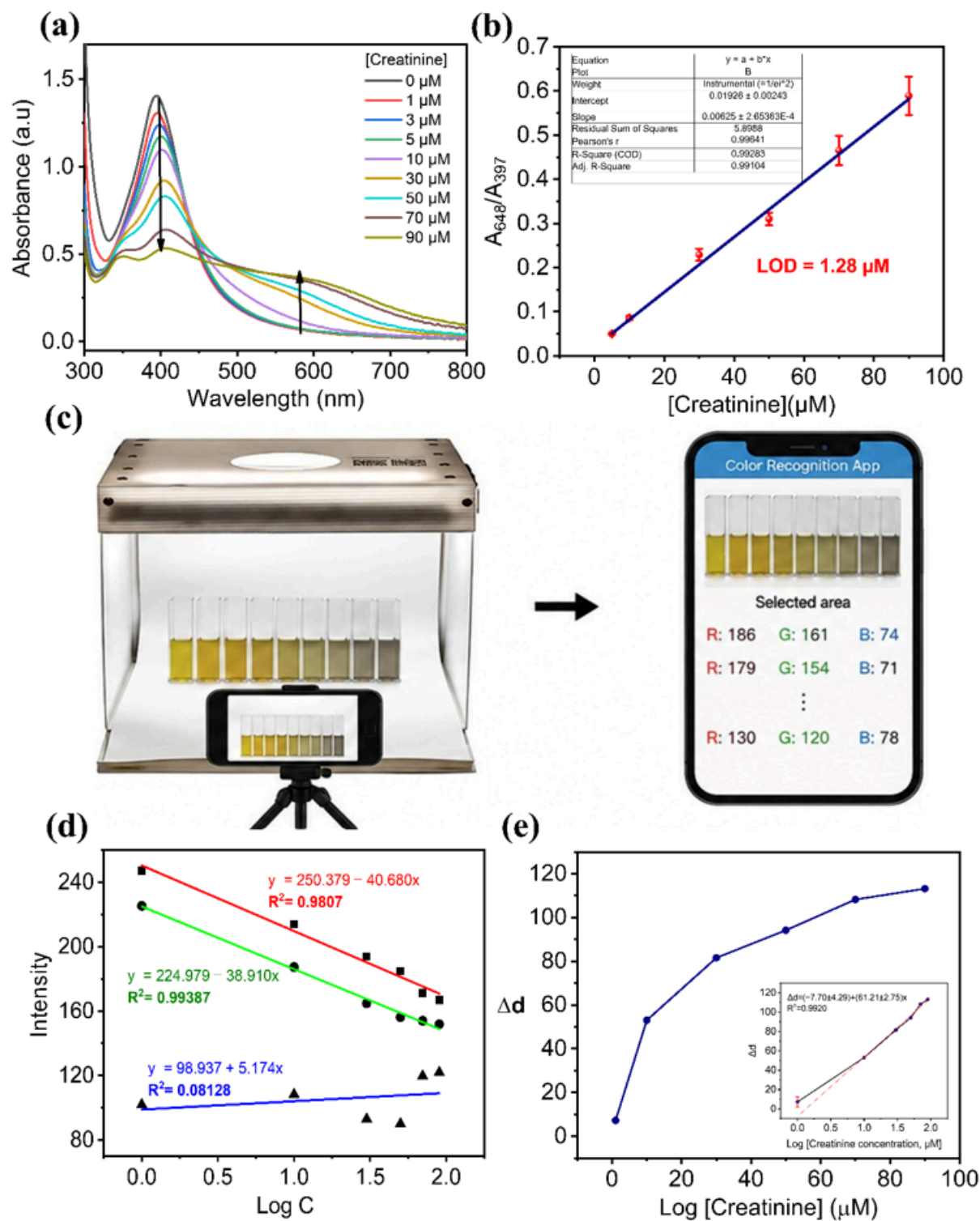


Figure 3

(a) UV-Vis absorption spectra of DTPA-capped AgNPs in the presence of different creatinine concentrations (0–90  $\mu\text{M}$ ), showing a gradual decrease in the SPR band at 398 nm and the formation of a broad band at longer wavelengths. (b) Spectrophotometric calibration curve based on the absorbance ratio ( $A_{648}/A_{397}$ ) versus creatinine concentration. (c) Schematic diagram of digital image analysis demonstrating the smartphone-based quantitative creatinine detection. (d) Linear regression plots of the RGB color channels against the logarithm of creatinine concentration, indicating that the green channel provides the highest correlation coefficient and analytical sensitivity. (e) Digital image colorimetric response expressed as  $\Delta d$  as

a function of creatinine concentration, with the inset showing the corresponding linear relationship between  $\Delta d$  and the logarithm of creatinine concentration.

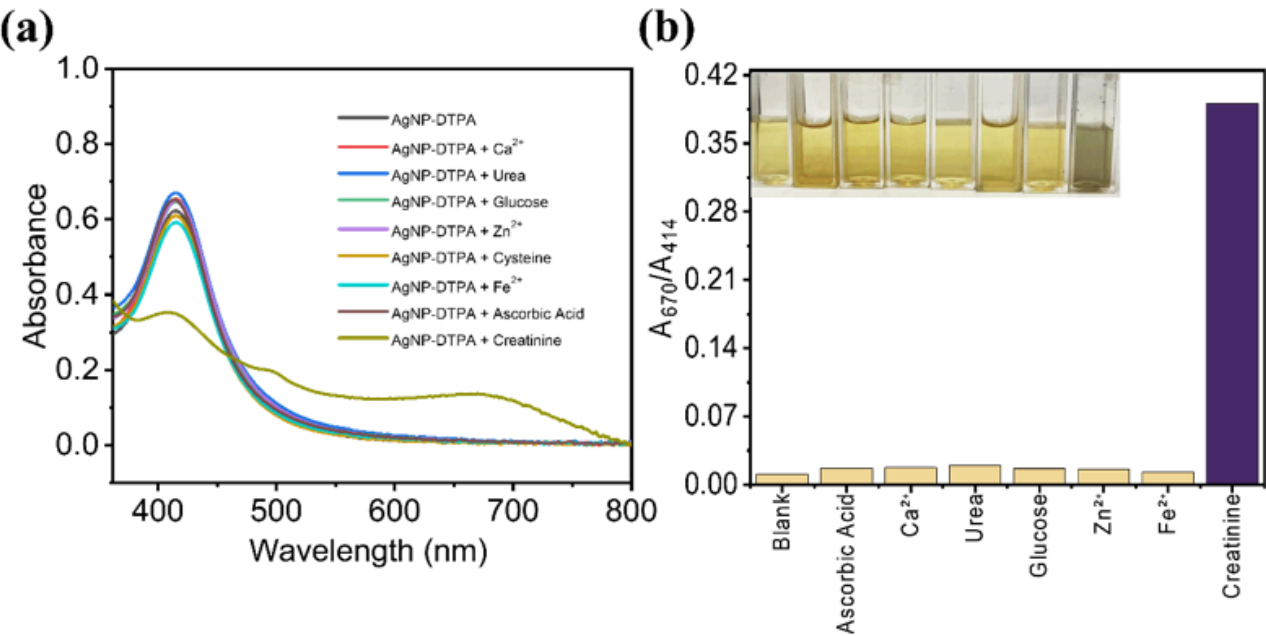


Figure 4

(a) UV–Vis absorption spectra of DTPA-AgNPs in the presence of various interfering species. A significant spectral change is observed only upon the addition of creatinine, indicating a selective interaction with the DTPA-AgNP system. (b) Selectivity of the DTPA-AgNPs assay toward creatinine, evaluated by the absorbance ratio ( $A_{670}/A_{414}$ ) after incubation with different inorganic and organic species (100  $\mu$ M) under alkaline conditions (pH 11).

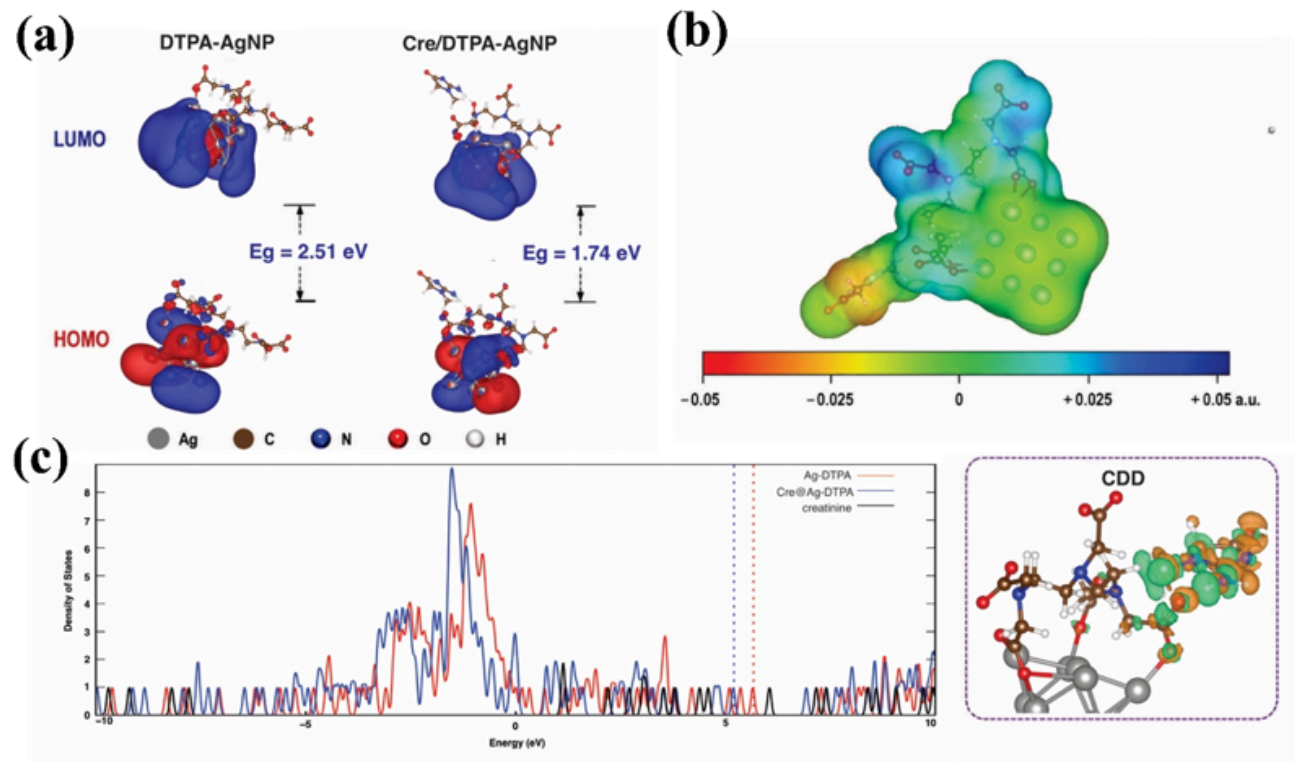


Figure 5

Computational analysis of the DTPA-AgNP/creatinine sensing system. (a) HOMO–LUMO isosurfaces of DTPA-AgNP and the DTPA-AgNP/creatinine complex. (b) Electrostatic potential (ESP) map of the optimized adsorption complex. (c) Density of states (DOS) and charge density difference (CDD, inset), showing electron redistribution upon creatinine adsorption.

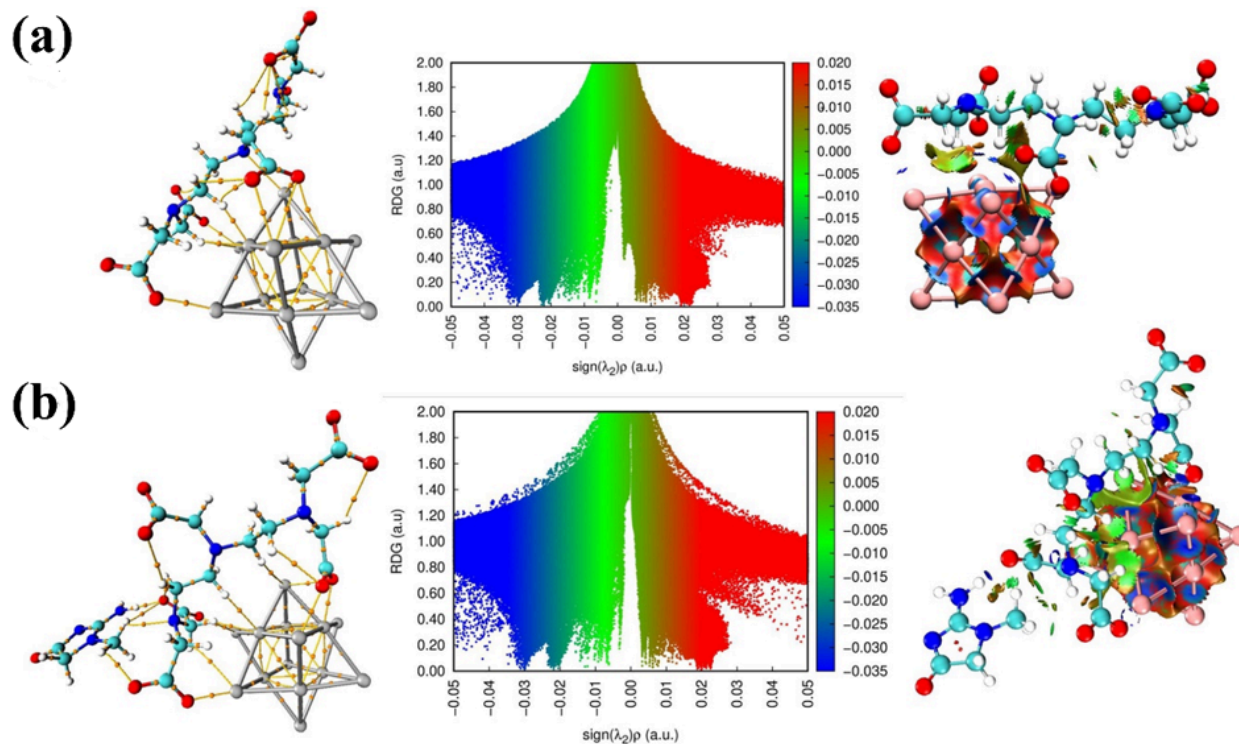


**Figure 6**

QTAIM and non-covalent interaction (NCI) analyses of (a) DTPA-AgNP and (b) the Cre/DTPA-AgNP complex. Optimized structures (left), RDG scatter plots (middle), and NCI isosurfaces (right) illustrate the intermolecular interactions governing creatinine adsorption.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupportingInformationPlasmonics.docx](#)
- [Scheme1.docx](#)