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Research Article

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Posted Date: October 19th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11242097/v1>

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Additional Declarations: No competing interests reported.

Green Rosemary Extract-Mediated Synthesis of Ag/Co/La₂O₃/Chitosan Nanocomposite for Multifunctional Antibacterial Activity and Simultaneous Removal of Binary Dyes from Water

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Abstract:

The development of multifunctional nanocomposites capable of simultaneously addressing chemical and biological water contaminants represents an important strategy for sustainable wastewater treatment. Herein, we report a novel Ag/Co/La₂O₃/chitosan nanocomposite designed for the simultaneous photocatalytic removal of methylene blue (MB) and crystal violet (CV) from a 1:1 binary dye system, coupled with antibacterial activity against representative Gram-negative and Gram-positive bacteria. Structure analysis showed that the synthesized nanomaterial consisted of multiple phases of Ag/AgCl, Co(OH)₂, La₂O₃, and La(OH)₃, with enhanced dispersion of nanoparticles and improved surface properties with the use of chitosan. The energy band gap reduced from 3.89 eV in Ag/Co/La₂O₃ NC to 2.94 eV in CS-NC, and there was an increase in the zeta potential from +8.19 to +13.78 mV. The CS-NC possessed excellent antibacterial properties and produced ROS in a dose-dependent manner, having a maximum inhibition zone (22 mm) against *S. epidermidis*. In binary MB and CV removal, 97.5 ± 0.7% and 94.0 ± 0.9% removal efficiencies were attained, respectively, using 0.50 g L⁻¹ of CS-NC under pH 9.0, 25 ± 1 °C, and 180 min under UV-A irradiation. Kinetic data were best described by the pseudo-second-order model (R² = 0.9998 for MB and 0.9996 for CV), while Langmuir analysis gave apparent maximum capacities of 75.925 and 75.062 mg g⁻¹. After seven cycles, 93.85% and 88.09% of the initial MB and CV removal efficiencies were retained, respectively. Limited Ag and Co release further supports its stability. Generally, this greenly fabricated multifunctional nanocomposite offers a promising, reusable, and environmentally relevant platform for integrated antimicrobial and dye-contaminated wastewater treatment.

Keywords: Ag/Co/La₂O₃/chitosan; Binary dye photocatalysis; Methylene blue-crystal violet; Antibacterial activity.

1. Introduction

The effluent of wastewater with dyed water from textile and allied industries is an ongoing problem for the environment as artificial dyes are stable chemically, highly colored even in small quantities, and sometimes difficult to degrade biologically. They cause adverse impacts on the aquatic environment due to light reduction and interference in the process of photosynthesis, whereas some of the dye molecules and their metabolites may have toxic properties [1,2]. Furthermore, the presence of dyes along with other contaminants present in solution form makes the situation even more complex and toxic. Recent research has shown that dyes along with their associated inorganic and organic contaminants have caused harmful impacts on the environment as well as on human health [3]. Despite the existence of many technological methods aimed at dye elimination, there is a tendency among laboratory investigations to perform experiments on separate dye solutions that fail to reflect the competitive situation that exists in natural wastewater samples. The effect of intermolecular interactions and competition of reactive or adsorptive centers can significantly change the effectiveness and kinetics of dye removal in mixed systems [4]. Biological, electrochemical, and oxidative methods might display different efficiencies depending on the type of dyes used [5,6]. Dye adsorption via various materials like magnetic chitosan and magnetite-composite ones has shown effectiveness in simultaneous removal of dyes; however, adsorption merely shifts the pollutants into another phase requiring some further treatment or even waste material management [7,8]. It makes sense to pay attention to multifunctional materials that could combine adsorption and photocatalytic destruction of dyes. Due to high surface area, controllable surface chemistry, and reactive species generation capability on exposure to light sources, nanostructured photocatalysts are regarded as one of the potential candidates for water purification applications [9]. Nanocomposites based on semiconductors could lead to the development of electron-hole pairs that react and contribute to the degradation of persistent organic substances. Nanostructures based on TiO_2 and ZnO have been thoroughly examined in the field of photocatalytic degradation of dyes; however, the actual performance of these structures may face challenges such as charge recombination, poor visible light absorption, and separation issues [10,11]. Utilization of various metals or metal oxides as components and embedding these structures within biodegradable or functional polymers could enhance the processes of charge separation, absorption, dispersion, and stabilization [12]. Therefore, advanced photocatalytic systems with complementary components are considered as one of the prospective approaches to achieving an effective treatment of dye-polluted wastewater [13].

Due to the possibility of both chemical contamination and presence of pathogenic microorganisms in wastewater, it would be beneficial to design multifunctional materials for water treatment able to degrade pollutants and inhibit bacteria. Multifunctional photocatalytic nanomaterials that demonstrate dye degradation and bactericidal activity at the same time reveal the possibility to carry out these two functions using different mechanisms [14]. Nanocatalysts containing silver are especially interesting due to their broad-spectrum bactericidal properties together with capability to tune charge transfer processes in photocatalysis [15]. As an example of multifunctional polymer-based material, ZnO -nanocomposites doped into chitosan matrices with

dye degradation and bactericidal properties can be presented [16]. La_2O_3 is a promising material for the construction of multifunctional nanomaterials due to the presence of both catalytic and antibacterial properties in La_2O_3 -containing structures along with their compatibility with chitosan, which enhances the surface functionality and interaction with pollutants [17]. Moreover, recent research works have shown the efficiency of dye degradation and antibacterial properties of chitosan-doped La_2O_3 nanostructures, confirming the use of these materials in the environmental protection field [18]. In addition, biodegradable polymer-based systems containing rare earth metals have been recently proposed to increase the rate of dye adsorption and degradation by using the combined effect of the inorganic active sites and polymers [19]. Thus, the addition of Ag and Co to La_2O_3 and chitosan can be a promising way to construct a multifunctional platform with the contribution of antibacterial activity and interfacial charge transfer provided by Ag, the ability to carry out photocatalytic redox reactions in the presence of Co-containing phases, and enhanced adsorption of chitosan.

Though much progress has been achieved in the field of photocatalytic nanomaterials, it should be mentioned that existing literature mainly concerns single-dye dye removal in solutions and little information is available regarding the co-treatment of dyes with different structures under competitive binary conditions. In addition, photocatalytic removal and antibacterial properties are often studied separately and not integrated into one treatment platform. Hence, we assume that the combination of Ag, Co, La_2O_3 , and chitosan would allow the creation of such properties as adsorption, photocatalytic, and antibacterial properties in one nanocomposite. Thus, in this research, Ag/Co/ La_2O_3 /chitosan nanocomposite is proposed to be created for the joint removal of methylene blue (MB) and crystal violet (CV) from the 1:1 binary solution, and the antibacterial activity of the nanocomposite will be tested on Gram-negative and Gram-positive bacteria. Different factors such as dosage, pH value, initial dye concentration, temperature, dark adsorption, and irradiation time will be studied, along with recyclability and mechanism of action.

2. Materials and Methods

2.1. Reagents and Chemicals

Silver nitrate (AgNO_3 , $\geq 99.9\%$), cobalt nitrate hexahydrate [$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\geq 98\%$], lanthanum oxide (La_2O_3 , $\geq 99.9\%$), chitosan (medium molecular weight, degree of deacetylation $\geq 75\%$) were of analytical grade and used without further purification. Methylene blue (MB, $\geq 95\%$) and crystal violet (CV, $\geq 90\%$) were used as model dyes. Certified Ag and Co standard solutions (1000 mg L^{-1}) were used for atomic absorption spectrometric calibration. All chemicals were obtained from reputable commercial suppliers and used as received; the chemical purity and manufacturer details are provided in Supplementary **Section S1**.

2.2. Instruments

The synthesized nanocomposites were characterized by UV-Vis, FTIR, HR-TEM, SEM-EDX, elemental mapping, XRD, and BET surface-area/porosity analyses. UV-Vis spectroscopy was also used for dye-removal measurements. Sonication and centrifugation were employed during nanocomposite preparation. Detailed

information on the instruments, manufacturers, operating conditions, and analytical procedures was provided in Supplementary **Section S1**.

2.3. Preparation of Rosemary extract

Leaves of rosemary were freshly collected, rinsed with tap and distilled water, and dried in the shade at room temperature without exposure to any direct sunlight. Dried leaves were ground using a laboratory mill. 10.0 g of plant powder was suspended in 100 mL of distilled water (1:10, w/v) and the mixture was soaked for 4 h at 30 °C. The mixture was filtered through muslin cloth and Whatman No. 1 filter paper [20]. The extract was stored in an amber-colored glass vial at 4 °C for no more than 48 hours and used for the preparation of nanocomposites.

2.4. Green Synthesis of Nanocomposite

Analytical-grade AgNO_3 , $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and La_2O_3 were used as Ag, Co, and La precursors, respectively. AgNO_3 (0.01 M) and $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.05 M) were separately dissolved in distilled water, and La_2O_3 (0.05 M) was dispersed in distilled water and added to the metal precursor solution while stirring continuously. Subsequently, an equal amount of freshly prepared rosemary extract was added slowly to the solution and stirred for 2 hours at 70 °C. After cooling to room temperature, the resulting suspension was centrifuged at 8000 rpm for 15 minutes, and the collected solid was washed three times with distilled water and once with ethanol. The obtained material was dried at 80 °C for 12 hours, ground with an agate mortar and pestle, and kept in a sealed glass vial.

2.4.1. Synthesis of Ag/Co/La₂O₃ Nanocomposite

The process of green synthesis of the Ag/Co/La₂O₃ NC required preparing a mixture of 100 mL aqueous solution of AgNO_3 (0.01 M), $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.05 M), and La_2O_3 (0.05 M) under stirring, followed by adding 100 mL of rosemary extract that had been recently prepared [21]. The mixture was stirred magnetically for 2 h at a temperature of 70 °C. After cooling to ambient temperature and centrifuging at 8000 rpm for 15 min, the obtained solid was washed with distilled water three times and once with ethanol. The product, dried at 80 °C for 12 h product was further ground and used for experiments.

2.4.2. Synthesis of Ag/Co/La₂O₃/Chitosan Nanocomposite

The functionalization of the chitosan was done using 1.0 g of chitosan dissolved 100 mL of 1.0% (v/v) aqueous acetic acid solution while stirring continuously for 12 h at room temperature, thereby forming a 1.0% (w/v) chitosan solution [22]. On the other hand, 1.0 g of Ag/Co/La₂O₃ NC was suspended in 100 mL of distilled water through ultrasonication for 30 min and then stirred for another 30 min. The nanocomposite suspension was slowly added to 100 mL of chitosan solution while stirring continuously for 3 h at 50 °C and approximately pH 5.0. The product was then cooled, centrifuged at 8000 rpm for 15 min, rinsed thrice with distilled water and once with ethanol, dried at 60 °C for 24 h, and crushed to form the Ag/Co/La₂O₃/chitosan NC, which was kept in a closed vessel until future use.

2.5. Agar well diffusion assay

The antibacterial activity of the Rosemary extract, Ag/Co/La₂O₃, and Ag/Co/La₂O₃/chitosan nanocomposites were determined by the agar well diffusion method using the selected bacterial strains [23]. Briefly, cultures of bacteria were prepared in sterile nutrient broth, and their optical density was set to around 0.5 McFarland Standard ($\approx 1 \times 10^8$ CFU mL⁻¹). Mueller-Hinton agar plates were evenly inoculated using a swab dipped in the bacterial culture solution. The agar plates were dried for 10 minutes before punching holes of 6 mm diameter using a sterile cork borer and subsequently filling them with 100 μ L of nanocomposite suspensions at various concentrations. Sterile distilled water and respective solvents were kept as negative controls, whereas a selected antibiotic standard was considered as a positive control. Incubation of the plates was done at 37 °C for 24 h, and the diameters of the zones of inhibition around the respective wells were measured in millimeters. All measurements were carried out in triplicate.

2.6. Dark Adsorption, Photocatalytic Experiments, Optimization, and Reusability

2.6.1. Dark Adsorption of MB and CV

The adsorption capacity of methylene blue (MB) and crystal violet (CV) onto the Ag/Co/La₂O₃/CS nanocomposite under dark conditions was investigated employing equimolar binary dye solutions consisting of 10 mg L⁻¹ MB and 10 mg L⁻¹ CV. For each test, 50 mL of dye solution was poured into a 100-mL Erlenmeyer flask, the nanocomposite was introduced at a dosage of 0.50 g L⁻¹, and the pH of the solution was set to 9.0 using dilute HCl or NaOH solutions. Then, the suspensions were stirred at 150 rpm for the temperature 25 ± 1 °C.

Aliquots were withdrawn after predetermined contact times of 0, 5, 10, 20, 30, 40, 50, and 60 min. Each aliquot was subjected to centrifugation to separate the nanocomposite from the suspension. The UV-Vis spectrophotometer measurements were carried out at the wavelengths of maximum absorption for MB and CV.

The percentage of dye adsorption was calculated as follows (Eq. 1):

$$\text{Adsorption (\%)} = \left(\frac{C_0 - C_t}{C_0} \right) \times 100 \quad \text{Eq. (1)}$$

where (C_0) and (C_t) are the initial and residual dye concentrations (mg L⁻¹), respectively. All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). The contact time at which the adsorption approached a constant value was considered the approximate dark-equilibrium time and was used in subsequent photocatalytic experiments.

2.6.2. Control Experiments

Control experiments were conducted to distinguish the contributions of direct photolysis, dark adsorption, and catalyst-assisted photocatalytic removal. Four experimental conditions were studied for the equimolar MB/CV binary solution system having concentrations of each dye of 10 mg L⁻¹: (i) dye solution without nanocomposite and without irradiation (dark stability control); (ii) dye solution without nanocomposite but under irradiation (photolysis); (iii) dye solution with 0.50 g L⁻¹ Ag/Co/La₂O₃/CS nanocomposite kept in the dark (dark adsorption); and (iv) dye solution with 0.50 g L⁻¹ nanocomposite under irradiation (photocatalytic process).

The starting pH value was 9.0, and the reaction temperature was set to 25 ± 1 °C. In the case of the irradiated reactions, the suspension was irradiated by UV-A LED light source (wavelength 365 nm, power 20 W) from a certain height of 10 cm above the reaction suspension. The irradiation intensity on the surface of the reactor was about 10 mW cm^{-2} . The suspension was continuously agitated at 150 rpm throughout the 180-min irradiation period. The reaction vessel was maintained under constant irradiation and temperature conditions during the experiments. Samples were collected before and after treatment, centrifuged to remove the nanocomposite, and analyzed by UV-Vis spectrophotometry.

The removal efficiency of MB and CV was calculated according to Equation (2):

$$\text{Removal (\%)} = \left(\frac{C_0 - C_t}{C_0} \right) \times 100 \quad \text{Eq. (2)}$$

where (C_0) is the initial dye concentration and (C_t) is the residual concentration after treatment at time (t), both expressed in mg L^{-1} . All control experiments were performed in triplicate.

2.6.3. Optimization of Photocatalytic Removal

Influence of irradiation time, dosage of nanocomposites, pH of initial solutions, initial dye concentration, and temperature on simultaneous MB and CV removal was carried out systematically [24]. All experiments were performed using 50 mL of a binary mixture of MB/CV with equal concentration of dyes, which was 10 mg L^{-1} , 0.50 g L^{-1} of nanocomposites, initial pH = 9.0, temperature = 25 ± 1 °C, rotation = 150 rpm, and irradiation time = 180 min.

For the experiment on irradiation time, suspensions were exposed to ultraviolet light irradiation for 0, 10, 20, 30, 60, 90, 120, 150, and 180 minutes. For the irradiation-time experiment, initial pH was set to 7.0, and the temperature was 25 ± 1 °C. For the nanocomposite dosage, the dosage of Ag/Co/La₂O₃/CS was changed from 0.10 to 1.50 g L^{-1} while keeping the concentration of dyes 10 mg L^{-1} . The initial pH was set to 7.0, and the temperature was 25 ± 1 °C. In the case of the initial-pH experiment, the pH was set at 3, 5, 7, 9, and 11 using dilute HCl or NaOH solutions. Nanocomposite concentration remained constant at 0.50 g L^{-1} while the temperature was held at 25 ± 1 °C.

In the case of the initial dye-concentration experiment, equal concentrations of MB/CV solutions were used at 5/5, 10/10, 20/20, 30/30, and 50/50 mg L^{-1} , while keeping a 1:1 ratio of MB. In addition, the nanocomposite concentration kept constant at 0.50 g L^{-1} , pH was 9.0, and the temperature was 25 ± 1 °C. For the experiment on temperature variation, the reaction temperature was varied to 20, 25, 30, 40, 50, and 60 °C while keeping the nanocomposite concentration at 0.50 g L^{-1} , pH constant at 9.0, and irradiation time at 180 min. Temperature was controlled using a thermostatically regulated system. After treatment, the samples were centrifuged and the residual concentrations of MB and CV were determined by UV-Vis spectrophotometry. The removal efficiency was calculated using Equation (2). All experiments were conducted in triplicate, and the results were expressed as mean $\pm$ SD.

2.6.4. Reusability and Photocatalytic Stability

The reusability of the Ag/Co/La₂O₃/CS NC was evaluated over seven consecutive photocatalytic cycles. For each cycle, 50 mL of an equimolar MB/CV solution containing 10 mg L⁻¹ of each dye was treated with 0.50 g L⁻¹ nanocomposite at pH 9.0 and 25 ± 1 °C under irradiation for 180 min with continuous agitation at 150 rpm. After each cycle, the nanocomposite was separated from the solution treated by centrifugation and recovered for subsequent reuse. The recovered material was washed thoroughly with distilled/deionized water to remove residual dye and loosely bound reaction products and then dried under the same conditions used for catalyst preparation/recovery before the next cycle. This procedure was repeated for seven consecutive cycles.

At the end of each cycle, the residual MB and CV concentrations were determined by UV-Vis spectrophotometry. The removal efficiency was calculated using Equation (2), whereas the retained activity was calculated relative to the first cycle according to Equation (3):

$$\boxed{\text{Retained activity (\%)} = \left(\frac{R_n}{R_1} \right) \times 100} \quad \text{Eq. (3)}$$

where (R_n) represents the removal efficiency (%) after the (n)th cycle and (R₁) represents the removal efficiency (%) obtained during the first cycle.

All reusability experiments were performed in triplicate. The variation in MB and CV removal efficiency over the seven consecutive cycles was used to evaluate the operational stability and reusability of the Ag/Co/La₂O₃/CS nanocomposite.

2.7. Silver and Cobalt Release Assay

Release of Ag and Co ions from the Ag/Co/La₂O₃/chitosan nanocomposite was performed by dispersing different amounts of the nanocomposite (0.10 to 1.00 g/L) in deionized water [25]. The suspensions were stirred at 37 ± 1 °C with constant agitation of 150 rpm for 24 h. After the specified time, the suspensions were centrifuged, and the supernatant was collected and filtered through 0.22-μm membrane filters. Release of Ag and Co ions from the Ag/Co/La₂O₃/chitosan nanocomposite was determined by an atomic absorption spectrophotometer (Buck Scientific, USA) with Ag and Co hollow-cathode lamps. Readings were recorded at 328.1 nm for Ag and 240.7 nm for Co. Calibration curves were prepared using certified Ag and Co standard solutions, and reagent blanks were analyzed in parallel. For quantification of the total amount of Ag and Co present, control samples with the same amount of nanocomposite were acid-digested with sulfuric acid under suitable conditions of digestion. The digested samples were appropriately diluted with deionized water and analyzed by atomic absorption spectrophotometry with the same wavelength settings. The acid-digested samples were used as the control samples for the total amount of extractable Ag and Co present in the nanocomposite, which was considered the reference value for percent metal released into the aqueous medium. All measurements were performed in triplicate, and the results were expressed as mg/L and percentage metal release.

2.8. Reactive oxygen species (ROS) generation assay

2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe was used to assess ROS generation by the Ag/Co/La₂O₃/chitosan NC [26]. Briefly, 1.0 mL of NC suspension at different concentrations (25, 50, 100, 200, 400, 800, and 1000 µg/mL) was mixed with 1.0 mL of DCFH-DA solution (10 µM) in phosphate-buffered saline (PBS, pH 7.4), giving a final reaction volume of 2.0 mL. The mixtures were incubated in the dark at 37 °C for 30 min to allow oxidation of DCFH-DA to fluorescent 2',7'-dichlorofluorescein (DCF). The fluorescence intensity was then measured using a fluorescence spectrophotometer at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. A DCFH-DA/PBS mixture without nanocomposite was used as negative control, while all samples were analyzed under identical conditions. ROS generation was expressed as relative fluorescence intensity compared with the control. Measurements were performed in triplicate.

2.9. Statistical Analysis

All tests were conducted in triplicate ($n = 3$), and values are given as means $\pm$ standard deviation (SD). All statistical tests were done using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA). Comparison between several groups of experiments was made using one-way analysis of variance (ANOVA) and the Tukey multiple comparison test. The level of significance was set at ($p < 0.05$). The linear and nonlinear regressions were used to study the kinetics of adsorption, the adsorption isotherms, and thermodynamics. The coefficient of determination (R^2) was used to evaluate the fitting of kinetic and isotherm models with comparison of calculated and experimental values. In all graphs, error bars show the standard deviation from three independent experiments. The level of significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Proposed Mechanism of Nanocomposites' Formation

The proposed green formation mechanism of the Ag/Co/La₂O₃ nanocomposite and its subsequent chitosan functionalization is illustrated in **Figure 1**. The phytochemicals present in rosemary extract, particularly phenolic, flavonoid, terpenoid, and other oxygen-containing compounds, are proposed to coordinate with metal species and facilitate the reduction and nucleation of Ag and Co-containing nanophases while simultaneously stabilizing the growing particles. Similar phytochemical-mediated reduction, complexation, nucleation, and stabilization mechanisms have been reported for plant-mediated Ag/Fe₂O₃ and Ag/Co nanocomposites [27]. Rosemary-derived biomolecules may additionally interact with the oxide surfaces through hydroxyl and carbonyl groups, promoting nucleation and limiting particle aggregation, as previously demonstrated for rosemary-mediated CuO-Fe₂O₃ nanocomposites [28]. In the proposed system, La₂O₃ provides an oxide support that facilitates the immobilization and dispersion of the Ag- and Co-containing phases, whereas the subsequent addition of chitosan promotes surface coating through interactions between its protonated amino groups and hydroxylated/oxygen-containing sites on the nanocomposite surface. This organic coating is expected to improve particle dispersion and aqueous stability while introducing additional surface functional groups. Thus,

as schematically presented in **Figure 1**, the overall process can be considered to involve phytochemical-assisted complexation and nucleation → formation/stabilization of Ag/Co/La₂O₃ nanostructures → chitosan adsorption/coating → formation of the final Ag/Co/La₂O₃/chitosan nanocomposite [27,28].

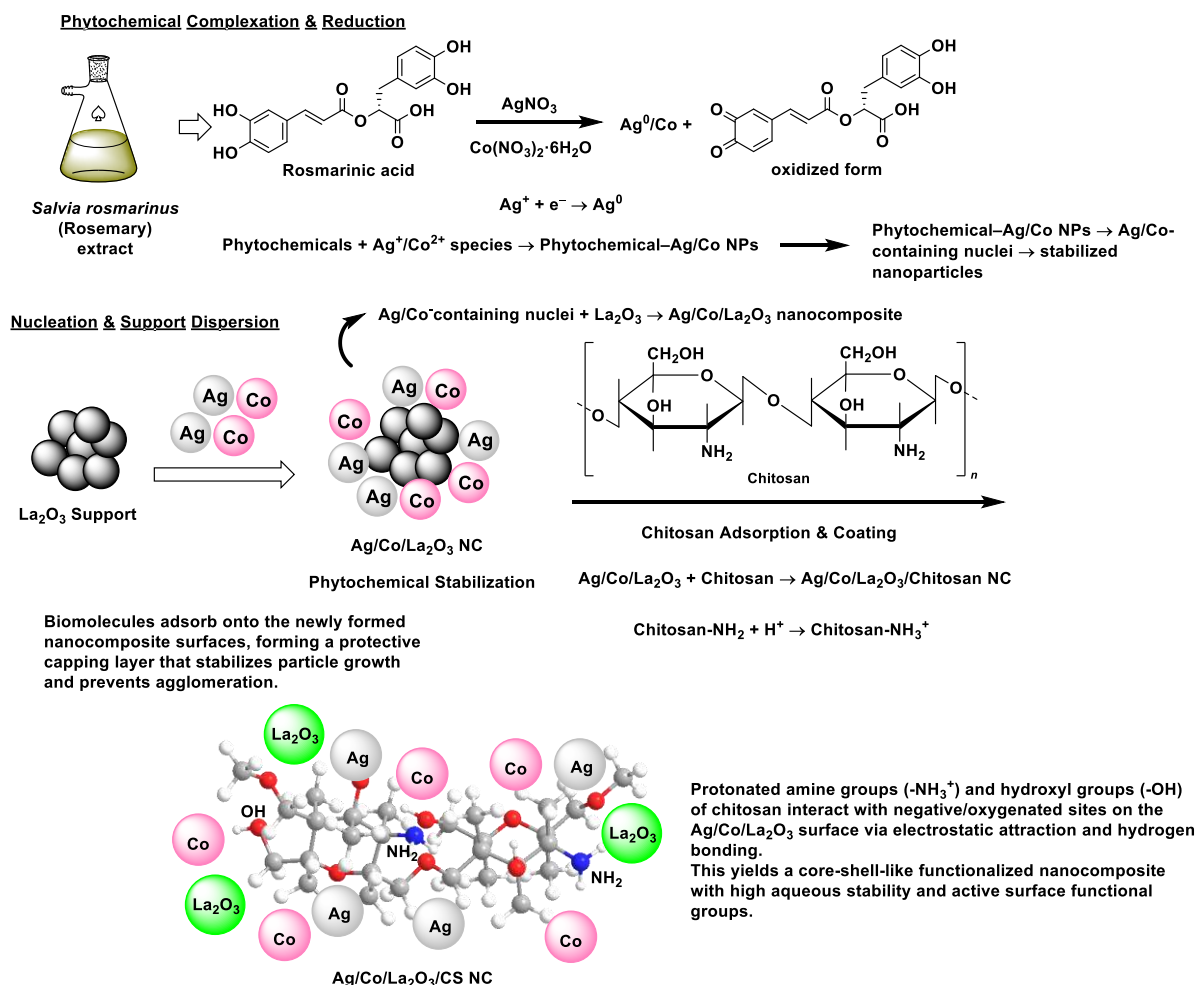


Figure 1. Proposed mechanism for the green synthesis of Ag/Co/La₂O₃ nanocomposite using rosemary extract and its subsequent chitosan functionalization, illustrating phytochemical-mediated complexation, nucleation, and stabilization of the Ag/Co-containing phases on the La₂O₃ support, followed by chitosan surface coating and formation of the final Ag/Co/La₂O₃/chitosan nanocomposite.

3.2. Characterization of Nanocomposites

3.2.1. UV-Vis Absorption Spectroscopy

The absorption properties of the rosemary extract, Ag/Co/La₂O₃ NC, and Ag/Co/La₂O₃/CS NC were studied through UV-Vis spectroscopy, and the spectra are shown in **Figure 2a**. The optical band gaps were determined from the linear portions of the Tauc plot $(\alpha h\nu)^2$ versus photon energy $(h\nu)$ (**Figure 2b**), and the results obtained are presented in **Table S1**. Rosemary extract showed strong absorption in the range of 280-330 nm attributed to $\pi \rightarrow \pi^*$ transition of phenolic compounds and $n \rightarrow \pi^*$ transition of carbonyl-containing compounds, such as rosmarinic acid, caffeic acid derivatives, and flavonoids. These phytochemicals act as primary

reducing/stabilizing agents that contribute to the synthesis of the nanocomposite due to electron donation through their hydroxyl and carbonyl functional groups. A similar UV-Vis absorption profile has been observed in rosemary extracts, containing phenolic compounds, which act as active molecules in the synthesis of nanoparticles [29].

After the synthesis of the nanocomposite, the Ag/Co/La₂O₃ NC displayed significant broadening of the absorption spectrum ranging from ultraviolet to visible light regions, showing successful incorporation of the metallic nanoparticles into the matrix of the host. The reason behind the enhanced absorption property is the contribution of the localized SPR effect of metallic Ag nanoparticles and ligand-to-metal charge-transfer between the O²⁻ and La³⁺ ions, together with the d-d transitions of the cobalt species. The broad absorption in the visible light shows effective photon absorption by the nanocomposite rather than the rosemary extract. Such enhancement of the visible-light absorption has previously been observed for the nanocomposites composed of Ag-decorated cobalt oxides where Ag nanoparticles play the role of electron sink and enhance the absorption spectra by their plasmonic effects [30]. Chitosan modification was also responsible for changes in the optical properties of the nanocomposite. It is clear from the absorption edge that the absorption range of the Ag/Co/La₂O₃/CS NC showed a higher broadness together with a red shift as compared to the uncoated NC, signifying more interaction among the amino and hydroxyl groups of chitosan with the inorganic NPs. The band-gap energy values derived from the Tauc plots of rosemary extract, Ag/Co/La₂O₃ NC, and Ag/Co/La₂O₃/CS NC were 1.76, 3.89, and 2.94 eV, respectively (**Table S1**). Even though the Tauc plot for rosemary extract gives the absorption edge of the mixture of phytochemicals, it is very clear from the comparison of both nanocomposites that the chitosan coating has decreased the band-gap energy by 0.95 eV. The decrease can be ascribed to the creation of electronic interface states that result from the strong interaction between the amino and hydroxyl functional groups of chitosan and the Ag/Co/La₂O₃ surface. This allows for the delocalization of electrons, decreases the gap between valence and conduction bands, and enables the absorption of visible light. Similar decreases in the gap energy have also been observed in the case of Ag/CoO/chitosan and cerium oxide/chitosan hybrid nanoparticles [30,31].

The lower energy gap of the Ag/Co/La₂O₃/CS NC will result in better photocatalytic activity due to efficient usage of photons of visible light. Under light irradiation conditions, electrons will be easily excited from the valence band to the conduction band; at the same time, Ag nanoclusters will act as electron traps that prevent the process of electron-hole pair recombination. At the same time, cobalt ions will be involved in fast redox reactions; besides, La₂O₃ will create a solid semiconductor structure with a high content of oxygen vacancies for charge transfer. The chitosan matrix will provide efficient dispersion of nanoparticles and the creation of an interconnected organic/inorganic interface facilitating charge transfer. Therefore, due to the synergistic effect of Ag, Co, La₂O₃, and chitosan, the formation of reactive oxygen species, such as •OH and •O₂⁻, will be promoted [30,31].

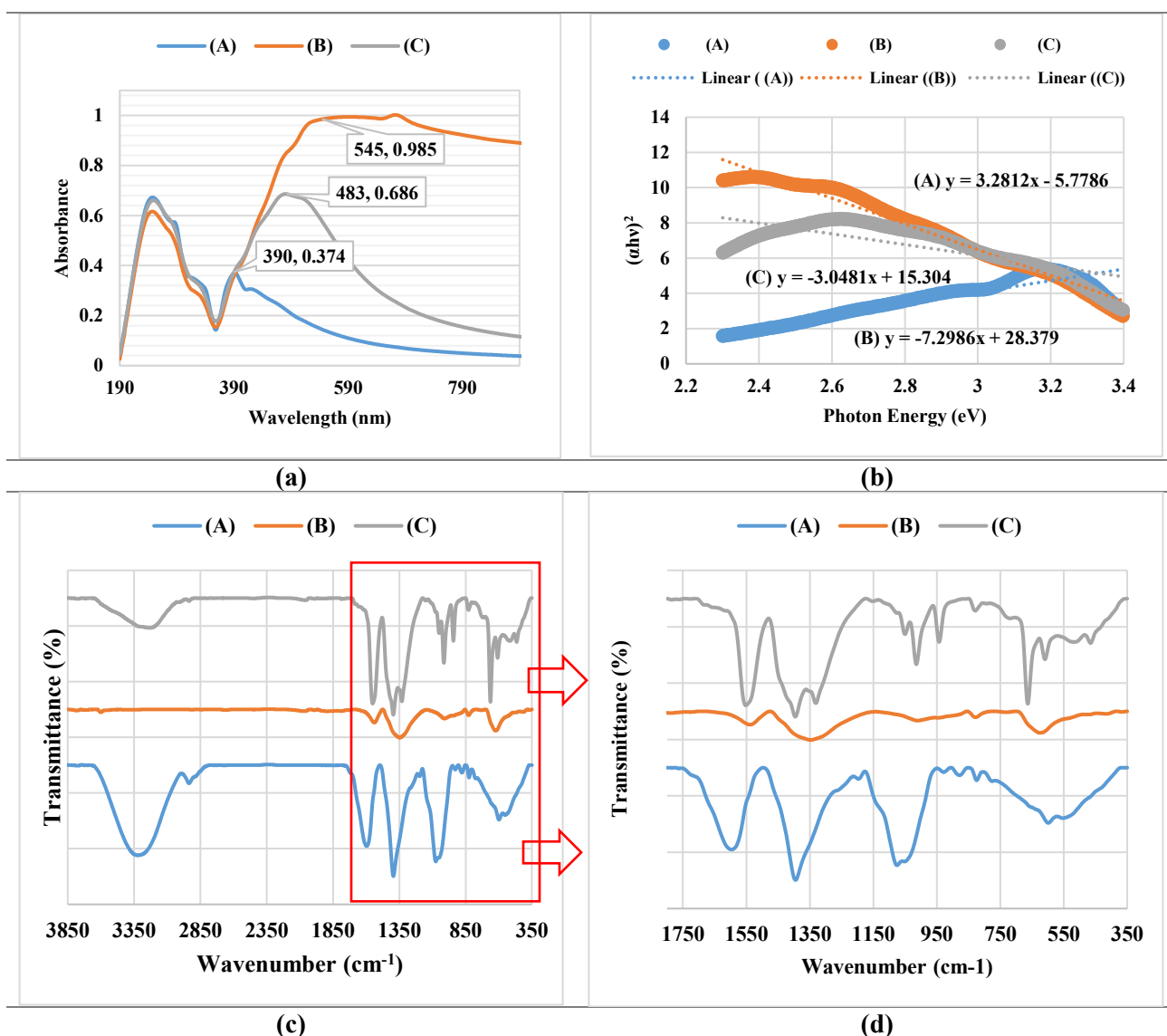


Figure 2. Optical and spectroscopic characterization of rosemary extract, Ag/Co/La₂O₃ nanocomposite (NC), and Ag/Co/La₂O₃/chitosan (CS) nanocomposite. (a) UV-Vis absorption spectra of (A) rosemary extract, (B) Ag/Co/La₂O₃ NC, and (C) Ag/Co/La₂O₃/CS NC, illustrating the changes in optical absorption following nanocomposite formation and chitosan functionalization. (b) Tauc plots $(\alpha h\nu)^2$ versus photon energy ($h\nu$) used to determine the direct optical band gap (E_g) of the synthesized materials. (c,d) FTIR spectra recorded over the 3850-350 cm⁻¹ region for (A) rosemary extract, (B) Ag/Co/La₂O₃ NC, and (C) Ag/Co/La₂O₃/CS NC, demonstrating the participation of phytochemical functional groups in nanoparticle synthesis and stabilization, as well as the successful incorporation of chitosan through characteristic vibrational band shifts and intensity variations.

3.2.2. FTIR Spectroscopic Analysis

The chemical interactions responsible for the biosynthesis and stabilization of the Ag/Co/La₂O₃ NC and Ag/Co/La₂O₃/CS NC were investigated using FTIR spectroscopy, and the corresponding spectra are presented in **Figure 2c,d**. The major vibrational bands and their assignments are summarized in **Table S2**. FTIR analysis of rosemary extract showed a wide band with an absorption maximum of 3383 cm⁻¹, representing O-H

stretching of phenolic hydroxyl groups along with N-H stretching of natural compounds. The significant bands at 2936 and 2880 cm^{-1} corresponded to asymmetrical and symmetrical C-H stretching of aliphatics, while those in the range of 1673-1539 cm^{-1} corresponded to C=O and C=C stretches of polyphenols and flavonoids, respectively. Further, the intense bands in the region of 1259-1027 cm^{-1} corresponded to C-O stretches of alcohols, ethers, and glycosidic linkage. These characteristic absorption bands agree well with previous ATR/FTIR investigations of rosemary extracts, which demonstrated that phenolic hydroxyl, carbonyl, and aromatic functional groups constitute the principal phytochemicals responsible for the antioxidant activity and reducing capability of rosemary during green nanoparticle synthesis [32].

Following nanocomposite formation, significant spectral changes were observed, confirming the interaction between rosemary phytochemicals and the inorganic components. The O-H stretching frequency range has been changed from 3383 cm^{-1} to 3548 cm^{-1} for the Ag/Co/La₂O₃ NC due to the presence of Ag, Co, and La ions in the complex, while the peak of aliphatic C-H stretching has been shifted to 2915 cm^{-1} . More importantly, new absorption frequencies were observed in the region below 700 cm^{-1} , namely 665, 630, 614, 529, 467, 408, and 366 cm^{-1} , corresponding to the lattice vibrations of La-O, Co-O, and Ag-O bonds. The emergence of these metal-oxygen vibrations provide strong evidence for the successful formation of the ternary Ag/Co/La₂O₃ nanocomposite. Similar FTIR features have been reported for Ag-decorated CoO nanocomposites, where the formation of metal-oxygen bonds and the disappearance or shifting of phytochemical functional groups confirmed the participation of plant metabolites during nanoparticle synthesis [30]. After coating with chitosan, additional spectral modifications confirmed the successful fabrication of the organic-inorganic hybrid nanocomposite. The broad O-H/N-H stretching band shifted to 3231 cm^{-1} , accompanied by characteristic amide absorption bands at 1559 and 1532 cm^{-1} , respectively, together with glucopyranose C-O-C stretching vibrations at 1051 and 1015 cm^{-1} , confirming the presence of the chitosan backbone surrounding the inorganic nanoparticles. Simultaneously, slight shifts in the metal-oxygen lattice vibrations to 664, 609, 507, and 465 cm^{-1} indicate strong coordination between the amino and hydroxyl groups of chitosan and the Ag/Co/La₂O₃ surface. The reaction takes place via hydrogen bonding and coordination of the lone pair of electrons from nitrogen and oxygen in chitosan with the metal ions on the surface. Similar spectral shifts have been observed in CoO/chitosan nanocomposites, where chitosan has successfully coordinated with cobalt oxide nanoparticles via amines, thereby ensuring stability of the nanoparticles [33].

Similarly, the outcomes of the FTIR studies also highlight the fact that the addition of chitosan not only acts as a coating on the surface of the nanocomposite material but also changes the interface chemistry via strong chemical interactions of the molecule with the inorganic matrix. Similar findings were found by researchers working on La₂O₃-chitosan hybrid materials, where the interaction between La₂O₃ and the amino and hydroxyl moieties of chitosan formed stable composite nanomaterials with high structural and physicochemical stability [34]. In the present investigation, the collective changes observed in the hydroxyl, amide, glucopyranose, and metal-oxygen vibrational groups confirm that initially the rosemary extracts were

responsible for the reduction and stabilization of Ag/Co/La₂O₃ nanoparticles, and the further modification by chitosan generated a strong hybrid compound due to hydrogen bonding, electrostatic interactions, and ligand-to-metal binding. These interactions at the interface will aid in nanoparticle dispersion, prevention of aggregation, interfacial electron transfer, and improved photocatalytic and antimicrobial activity of Ag/Co/La₂O₃/CS nanocomposite.

3.2.3. Transmission Electron Microscopy (TEM) Analysis

Morphology, particle size, and crystallinity of the synthesized Ag/Co/La₂O₃ NC and Ag/Co/La₂O₃/CS NC were studied using transmission electron microscopy (TEM) and are shown in **Figure 3**. The Ag/Co/La₂O₃ NC (**Figure 3a,b**) had mostly spherical and quasi-spherical nanoparticles, which were partially agglomerated to interconnected aggregates. Such agglomeration is common to metal oxide nanocomposites synthesized by greener methods due to the high surface energy of nanosized particles and attractive van der Waals forces between adjacent nanoparticles formed during synthesis and drying processes [35,36]. Individual particles had a size ranging from 23.74 to 73.74 nm, suggesting uniform nucleation and well-controlled crystallization. The presence of separate particles and dense aggregates indicates the simultaneous role of phytochemicals extracted from the plant in reduction and stabilization of nanoparticles [36]. Nanoscale size provides a high surface-to-volume ratio that is necessary for improvement of photocatalytic properties due to an increasing number of active sites.

After the addition of chitosan, dramatic changes in morphology were noted (**Figure 3d,e**). The Ag/Co/La₂O₃/CS NC formed a hybrid structure with uniformly distributed nanoparticles inside elongated nanorods and nanotubes. The sizes of the nanostructures varied between 19.17 nm and 68.87 nm, which indicates that chitosan limited particle size growth and helped achieve anisotropic structures. In comparison with the bare Ag/Co/La₂O₃ NC, the NC modified with chitosan had smaller agglomerations and better particle dispersion. This may be due to the high concentration of amino and hydroxyl groups present in chitosan, which interact with Ag, Co, and La ions *via* electrostatic and hydrogen bonds, which sterically stabilize particles and prevent their excessive agglomeration [37,38]. Also, the formation of elongated structures helps increase the surface area and electron transport pathways. The SAED patterns of the nanocomposites (**Figure 3c,f**) showed bright concentric diffraction rings made up of distinct diffraction spots, thus proving their polycrystalline structure. The clearly visible diffraction rings reveal the high crystallinity of the metal and oxide phases, while the diffraction rings' morphology reveals the randomness of the alignment of multiple nanocrystals. It is worthy of mention that the SAED pattern of the Ag/Co/La₂O₃/CS NC maintained its clarity even after coating with a polymer, which reveals that chitosan had not changed the crystalline structure of the inorganic nanocomposite; rather, it acted as an outer protective layer for the inorganic nanocomposite. It is very important to maintain the crystallinity of the material after coating since crystalline regions facilitate charge separation and prevent electron-hole recombination [38]. In summary, TEM analysis indicates that the role of chitosan extends beyond being a mere stabilizer to one of being a morphological directing agent as it facilitates conversion of

nanoparticle clusters to a more uniformly dispersed hybrid nanostructure. This structural modification is anticipated to facilitate better exposure of active sites, increased surface area, and charge transfer reactions.

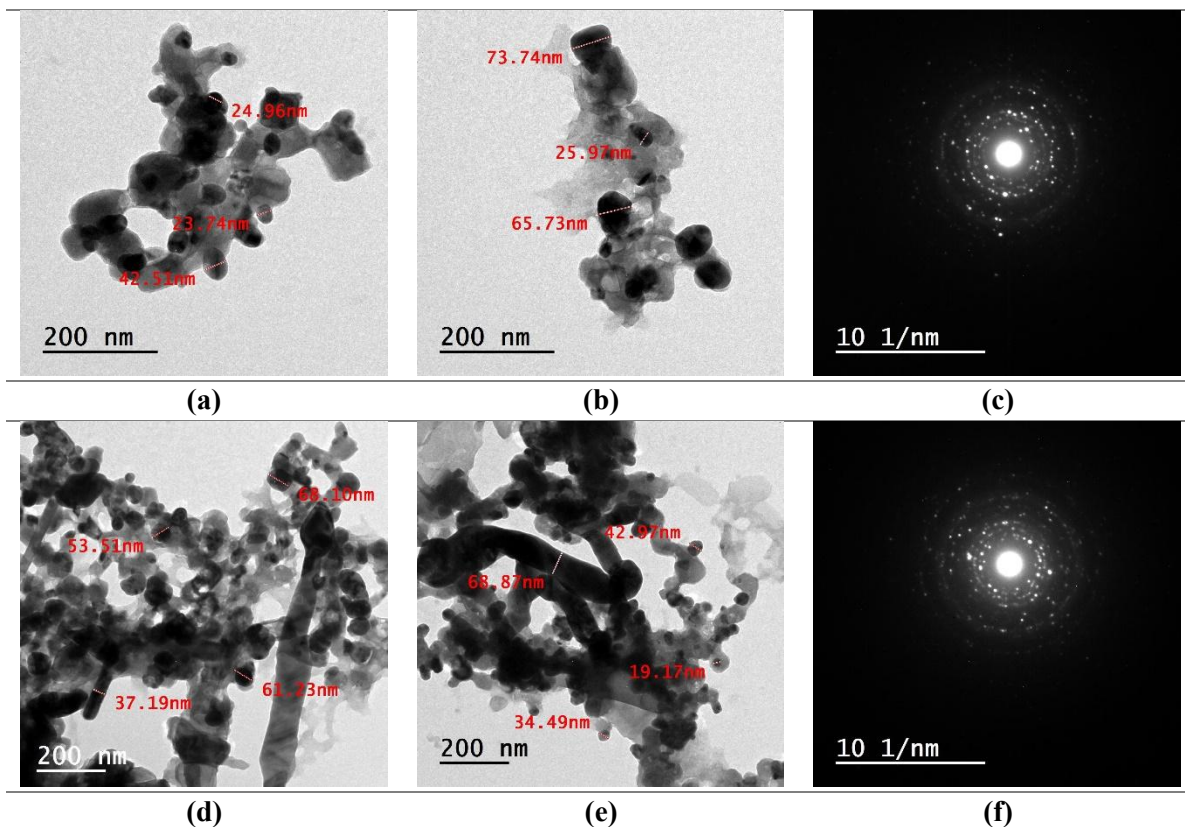


Figure 3. Transmission electron microscopy (TEM) characterization of the synthesized nanocomposites. (a,b) TEM images of Ag/Co/La₂O₃ NC showing spherical to quasi-spherical nanoparticles with particle sizes ranging from **23.74 to 73.74 nm**. (c) SAED pattern confirming the polycrystalline nature of Ag/Co/La₂O₃ NC. (d,e) TEM images of Ag/Co/La₂O₃/CS NC showing uniformly dispersed nanoparticles embedded within elongated rod-/tube-like chitosan-supported structures with dimensions ranging from **19.17 to 68.87 nm**. (f) SAED pattern confirming the preservation of crystallinity after chitosan functionalization.

3.2.4. Zeta Potential Analysis

The surface charge characteristics and colloidal stability of the synthesized Ag/Co/La₂O₃ NC and Ag/Co/La₂O₃/CS NC were evaluated by zeta potential measurements using laser Doppler electrophoresis, and the corresponding electrophoretic parameters are summarized in **Tables S3** and **S4**, while the zeta potential distributions are presented in **Figure 4**. Surface charge is one of the most important physicochemical parameters governing nanoparticle dispersion, aggregation behavior, suspension stability, and interactions with biological membranes and pollutant molecules [39,40]. The Ag/Co/La₂O₃ NC exhibited an average zeta potential of +8.19 mV with an electrophoretic mobility of $+0.57 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The positive value of the surface charge implies that the surface of the nanoparticles has been protonated during the green synthesis procedure, hence maintaining a positive charge of the surface hydroxyls in addition to having the phytochemical capping agents from the rosemary extract. Although the value of the zeta potential is relatively

lower than that of highly stable colloid systems ($\zeta \geq 30$ mV), it provides evidence that there is indeed a positively charged colloid system with moderate stability. The frequency shift of 1.11 Hz obtained with the sample peak frequency being 261.23 Hz in relation to the reference frequency of 260.12 Hz also provides evidence that the nanocomposite is positively electrophoretically mobile. Similar low positive zeta potential values have been reported for green-synthesized metal oxide nanocomposites, where surface phytochemicals provide partial electrostatic stabilization while preserving reactive surface sites required for catalytic applications [40,41].

Following chitosan functionalization, a remarkable increase in the positive surface charge was observed. The Ag/Co/La₂O₃/CS NC exhibited a zeta potential of +13.78 mV, accompanied by an increase in electrophoretic mobility to $+0.96 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. This enhancement represents an increase of approximately 68% compared with the pristine Ag/Co/La₂O₃ NC, demonstrating the significant influence of the chitosan coating on the surface electrochemical properties. The positive charge could be attributed to the protonated amino groups ($-\text{NH}_3^+$) that exist in great amounts in chitosan and are positively charged in the aqueous medium, being effectively adsorbed on the nanoparticle surface by complexation with Ag, Co, and La oxides. As a result, the presence of such a polymer coating increases electrostatic repulsion of the adjacent nanoparticles and ensures their better colloidal stability. An increase of the same magnitude in zeta potential values has been observed after chitosan treatment of Ag-decorated CoO nanoparticles and Co₃O₄/chitosan nanocomposites due to the presence of protonated amino groups acting as effective stabilizing centers [30,42].

The improvement in surface charge is consistent with the TEM and SEM observations, which revealed reduced nanoparticle aggregation and a more homogeneous particle distribution after chitosan incorporation. The higher positive zeta potential also agrees with the FTIR results, where characteristic amino vibrations confirmed successful interaction between chitosan and the Ag/Co/La₂O₃ surface. Although the measured zeta potential values remain below the conventional threshold for highly electrostatically stabilized suspensions, the combined contribution of electrostatic repulsion and steric stabilization imparted by the chitosan polymer is expected to produce substantially improved colloidal stability. Chitosan chains surrounding the nanoparticles generate a protective steric barrier that minimizes particle-particle collisions even when the absolute zeta potential is relatively moderate [42,43]. The higher surface charge and colloidal stability of the Ag/Co/La₂O₃/CS NC are anticipated to directly impact the photocatalytic and biological efficiency of the NCs. Better dispersion of the nanoparticles allows an increasing available surface area of the nanoparticles and number of catalytically active sites, which would help in the better adsorption of contaminating molecules and formation of reactive oxygen species under visible light irradiation. In addition, a positively charged chitosan coating of the NCs is expected to improve the electrostatic interaction between the NCs and negatively charged bacterial cell walls as well as other anionic contaminants, resulting in improved antibacterial and catalytic efficiencies. Thus, the zeta potential measurements provide compelling evidence of successful surface modification of the Ag/Co/La₂O₃ nanocomposites using chitosan.

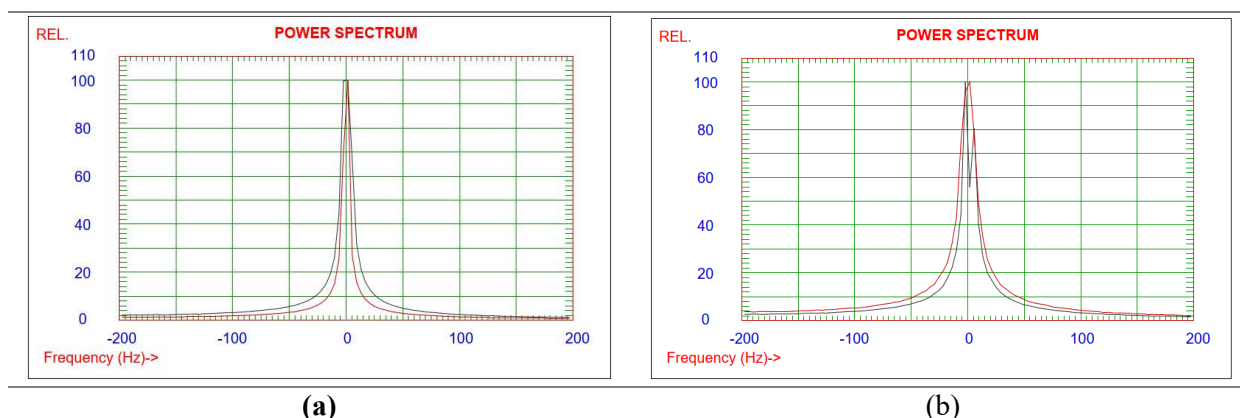


Figure 4. Zeta potential analysis of the synthesized Ag/Co/La₂O₃ nanocomposites. (a) Zeta potential distribution of the Ag/Co/La₂O₃ NC showing an average surface charge of +8.19 mV and moderate colloidal stability. (b) Zeta potential distribution of the Ag/Co/La₂O₃/CS NC exhibiting an increased positive surface charge (+13.78 mV) after chitosan functionalization, indicating enhanced electrostatic stabilization and improved colloidal dispersion resulting from the protonated amino groups of the chitosan matrix.

3.2.5. Scanning Electron Microscopy (SEM) Analysis

The surface morphology and microstructural architecture of the synthesized nanocomposites were further examined by SEM, as illustrated in **Figure 5**. The Ag/Co/La₂O₃ NC (**Figure 5a,b**) exhibited a highly compact granular morphology composed of densely packed submicron aggregates with irregular grain boundaries. Individual nanoparticles were strongly fused into larger secondary clusters, producing rough surfaces and randomly distributed interparticle pores. Such compact aggregation is characteristic of metal oxide nanocomposites synthesized by green methods because nanoparticle growth and drying promote particle coalescence through interparticle attractive forces [35,36]. At higher magnification (30,000×), rough grain surfaces and interconnected porous channels became more evident, whereas lower magnification (15,000×) revealed larger agglomerated microclusters distributed throughout the sample. Although aggregation reduces the accessible surface area, the interconnected pores can facilitate the diffusion of pollutant molecules toward catalytically active sites.

A fascinating change in the morphological pattern was achieved after the coating of chitosan (**Figures 5c and d**). The Ag/Co/La₂O₃/CS nanocomposite showed a heterogeneous three-dimensional structure composed of flake-, sheet-, ribbon-, and rod-shaped crystallites within the polymer matrix. In contrast to the densely packed pattern of the as-synthesized nanocomposite, the chitosan-coated one has an open, porous, and highly interconnected network that is characterized by less nanoparticle agglomeration. Such an effect is caused by the good film-forming properties of chitosan due to its amino and hydroxyl groups, which effectively interact with the surfaces of nanoparticles and prevent their uncontrollable aggregation [37,38]. The resulting rough and porous surface provides abundant exposed edges and adsorption sites, thereby facilitating reactant diffusion and enhancing catalyst-pollutant interactions. The results of SEM analysis corroborate well with those from TEM analysis and indicate that chitosan can alter the surface morphology without affecting the

crystalline inorganic lattice. The presence of hierarchical pores is especially beneficial for photocatalysis since it increases the absorption of light due to the multiple scattering of light rays, minimizes the path of diffusion of photoexcited charge carriers, and promotes reactive oxygen species formation on the surface of the catalyst [36]. Besides, the increase in the surface area and pores is also expected to promote the interaction between the nanocomposite and the cell membrane of microorganisms.

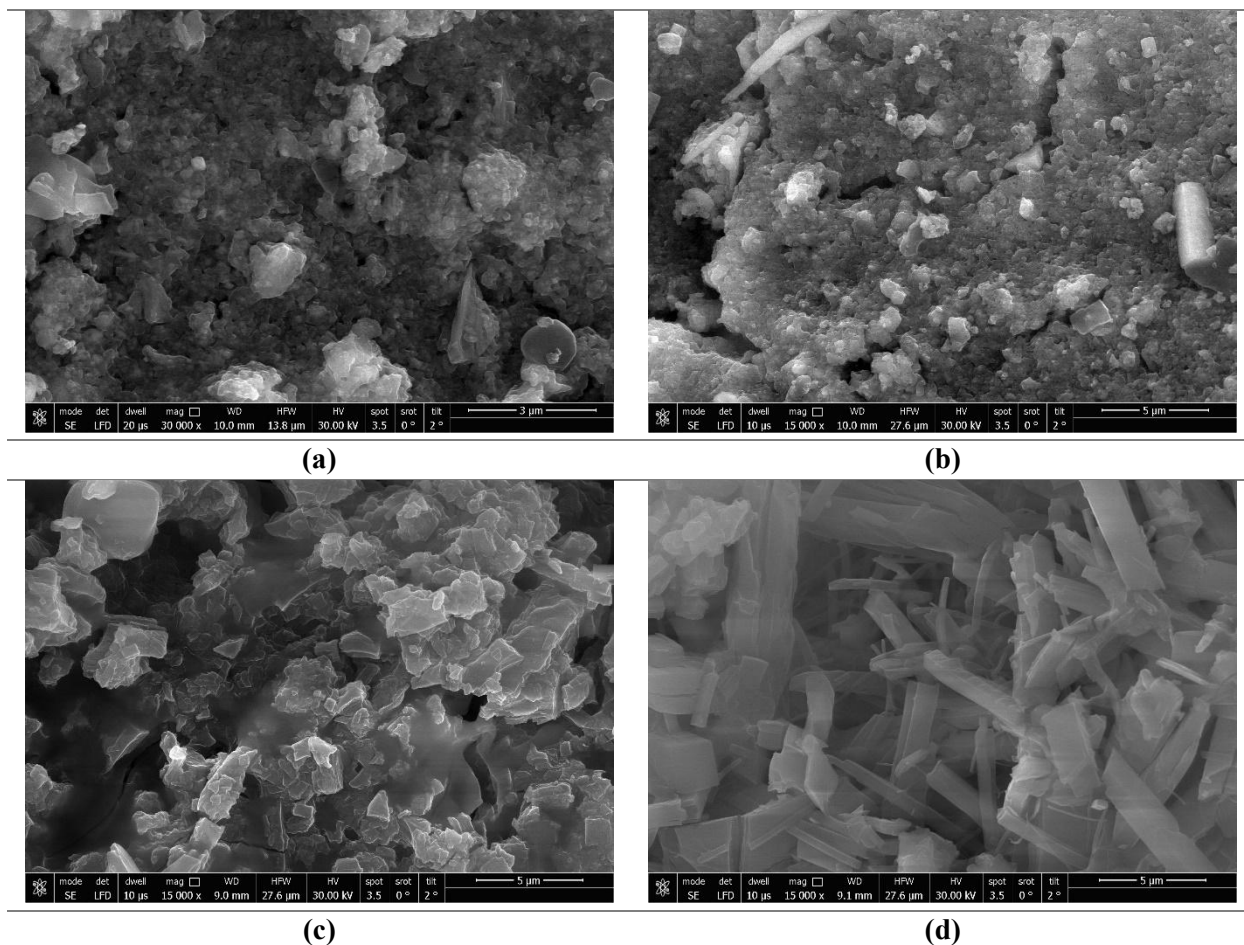


Figure 5. Scanning electron microscopy (SEM) micrographs of the synthesized nanocomposites. (a,b) Ag/Co/La₂O₃ NC exhibiting densely packed granular aggregates with rough surfaces and interconnected pores. (c,d) Ag/Co/La₂O₃/CS NC showing a hierarchical porous morphology composed of stacked flakes, sheet-like structures, and elongated rod-/ribbon-like crystallites, demonstrating the morphology-directing effect of chitosan.

3.2.6. EDX and Elemental Mapping Analysis

The elemental composition and elemental distribution of the prepared Ag/Co/La₂O₃ and Ag/Co/La₂O₃/CS nanocomposites were studied by EDX spectroscopy and elemental mapping (**Figs. 6 and 7; Tables S5 and S6**). From the EDX spectrum of Ag/Co/La₂O₃ NC, characteristic peaks of O, Ag, La, and Co were seen, indicating that all the desired inorganic elements were present in the NCs. The compositions of the material were 31.52 wt% O, 37.29 wt% Ag, 28.87 wt% La, and 2.33 wt% Co. The low Co content is since it was deliberately added

as an additional element. There were no unexpected elemental peaks in the spectra, confirming the preparation of the Ag/Co/La₂O₃ NCs. After the chitosan modification process, the C and N signals appeared in the EDX spectrum, thus proving the presence of the chitosan layer in the material. In the Ag/Co/La₂O₃/CS NC, the content of C, N, O, Ag, La, and Co was 1.10 wt%, 0.832 wt%, 39.13 wt%, 5.72 wt%, 36.89 wt%, and 8.85 wt%, respectively. The reduction in the relative Ag signal and the simultaneous appearance of C and N signals are typical results for surface coverage with the organic polymer. The increase in the relative amount of Co could be caused by the alteration in the sample size and matrix effect after the chitosan modification process. Thus, the values from the EDX analysis should only be treated semi-quantitatively.

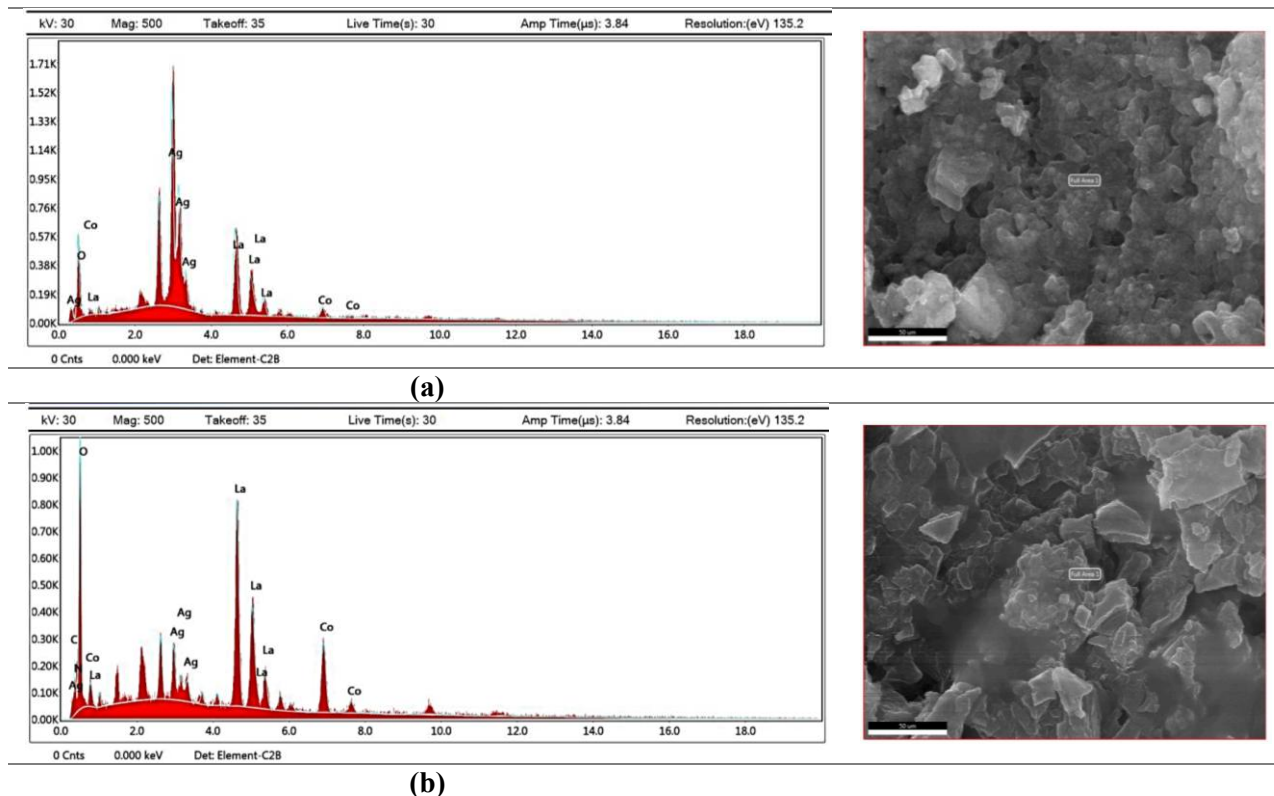
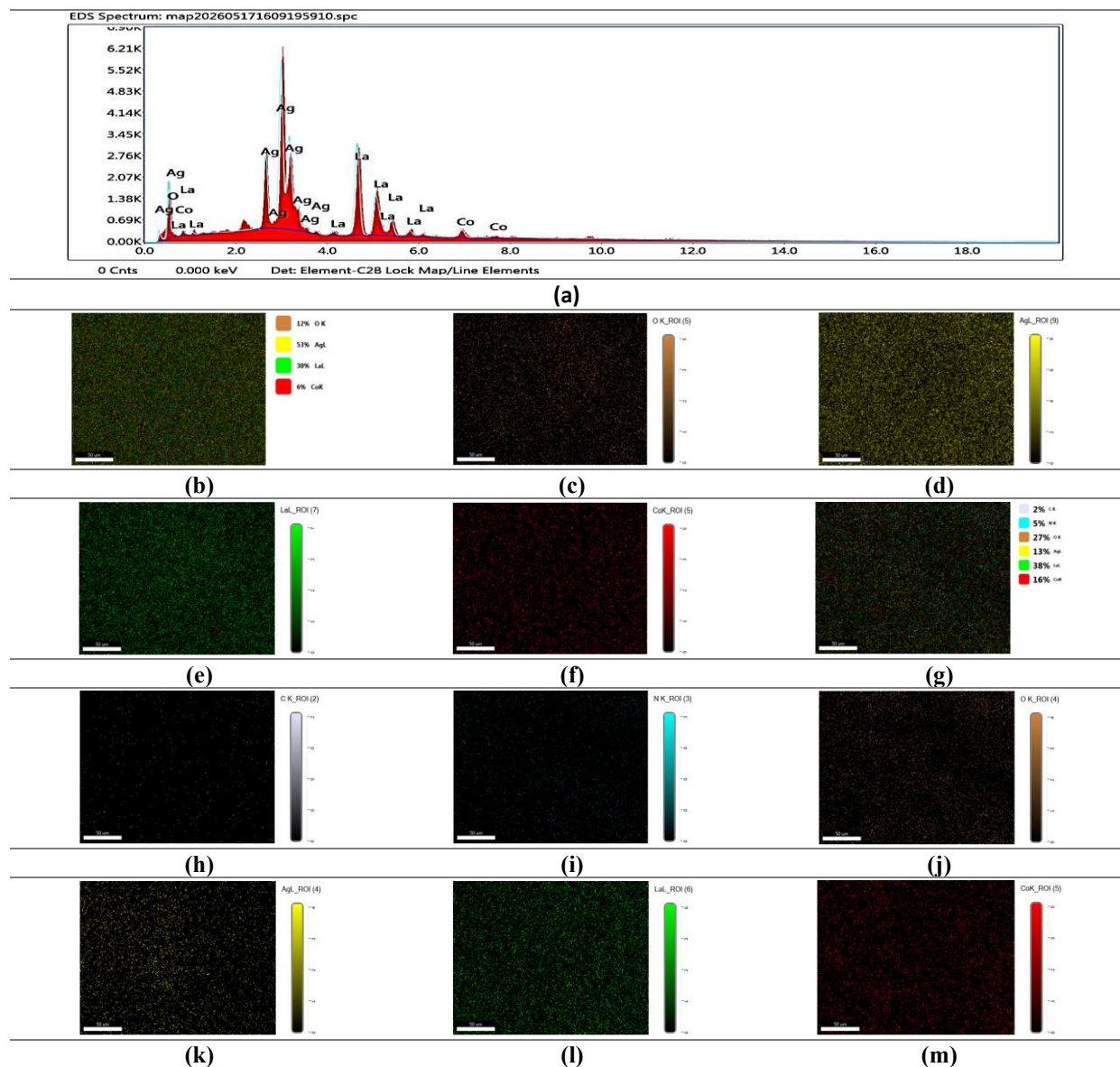


Figure 6. EDX spectra of (a) Ag/Co/La₂O₃ NC and (b) Ag/Co/La₂O₃/CS NC, confirming the characteristic elemental signals of the inorganic constituents and the appearance of C and N after chitosan functionalization.

Further, elemental mapping proved the spatial presence of the inorganic compounds in the material. In the case of Ag/Co/La₂O₃ NC, O, Ag, La, and Co were present across the selected area with contributions from mapping of 12%, 53%, 30%, and 6% respectively (**Table S6**). The contribution from Ag element was the highest and exhibited homogeneous spatial presence. On the other hand, La and O elements were highly spatially correlated due to La₂O₃ structure. Co element was evenly distributed at low intensity without any sign of clustering. The mapping for Ag/Co/La₂O₃/CS NC revealed the presence of extra elements, namely C and N, where the mapping contributions were 2% and 5%, respectively, which confirmed the presence of chitosan. Oxygen was still highly distributed (27%), while the highest mapping contribution (38%) was achieved for La, followed by Ag (13%) and Co (16%). Since the mapping showed the La and O elements being present in the form of oxide,

their spatial distribution confirmed the maintenance of the oxide framework despite the polymer modification, whereas the relatively homogeneous distributions of the elements Ag and Co indicated that the corresponding metal-containing materials were still dispersed in the chitosan-based matrix. The absence of pronounced elemental segregation further suggests that chitosan functionalization did not induce substantial phase separation or macroscopic agglomeration.



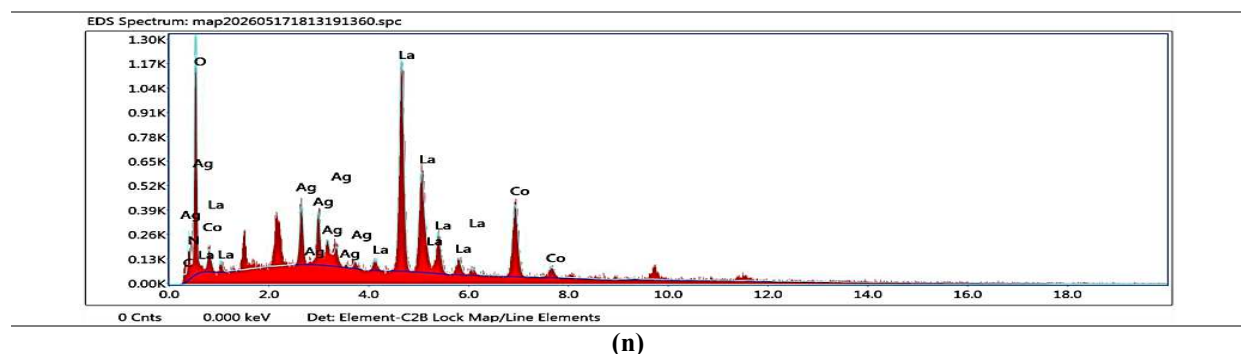


Figure 7. Elemental mapping analysis of (a-f) Ag/Co/La₂O₃ NC and (g-n) Ag/Co/ La₂O₃/CS NC. (a,n) EDS sum spectra recorded during elemental mapping, showing the characteristic X-ray energy emissions and peak distributions of the detected elements; (b,g) elemental composition/overlay maps; (c,j) O; (d,k) Ag; (e,l) La; (f,m) Co; and (h) C and (i) N. The elemental maps demonstrate the spatial distribution and dispersion of the constituent elements throughout the nanocomposites.

3.2.7. XRD Analysis

The XRD spectra of the green synthesized Ag/La₂O₃ NC and Ag/Co/La₂O₃/CS NC are shown in **Figure 8**, while the XRD peak indexing and phase quantification of those are provided in **Tables S7** and **S8**. In case of XRD spectra of Ag/La₂O₃ NC (**Fig. 8A**), it was found that the material is made up of multiphase crystalline structures. The peaks at $2\theta = 27.94^\circ, 32.35^\circ, 46.33^\circ, 54.91^\circ, 57.55^\circ$, and 76.80° are due to cubic AgCl, while $38.22^\circ, 44.39^\circ, 64.54^\circ$, and 77.49° peaks correspond to the face-centered cubic Ag. In addition, La containing phases include hexagonal La(OH)₃ and La₂O₃ having reflections at $15.79^\circ, 27.40^\circ, 31.74^\circ, 39.60^\circ$, and 48.78° for La(OH)₃ and at 29.49° for La₂O₃. These assignments agree with the corresponding ICDD/PDF standards listed in **Table S7** and confirm the coexistence of Ag-, La₂O₃-, and La(OH)₃-derived crystalline domains.

Remarkably, AgCl was the major component, contributing 88.7 wt% content, followed by silver (Ag), 5.1 wt%; La(OH)₃, 4.7 wt%; and La₂O₃, 1.5 wt%. The co-existence of Ag and AgCl indicates that the silver precursor was partially reduced during the synthesis process, whereas other forms of Ag underwent reaction with the chloride ions to give the formation of crystalline AgCl. Formation of multiple phases is expected in plant-assisted synthesis due to phytochemicals having the ability to reduce metal ions, form complexes, and stabilize surfaces at the same time. Formation of La(OH)₃ with La₂O₃ might also indicate the hydroxylation/hydration of lanthanum oxide surfaces. Phase-dependent structure has been observed before in chitosan/La₂O₃ nanocomposites [44-46]. After the incorporation of Co and chitosan, the diffraction pattern has become much more complicated, which demonstrates that Ag/Co/La₂O₃/CS hybrid has been formed successfully (**Fig. 8B**; **Table S8**). Along with the previously detected peaks belonging to AgCl, Ag, La₂O₃, and La(OH)₃, there have appeared additional diffraction peaks of hexagonal Co(OH)₂ at $2\theta = 20.17^\circ, 36.81^\circ, 38.96^\circ, 50.67^\circ, 61.80^\circ$, and 68.43° . The new phase containing chitosan has been determined as chitosan L-ascorbate, characterized by significant reflections at $7.80^\circ, 9.05^\circ, 18.63^\circ$, and 35.45° . The appearance of low-angle reflections typical for the chitosan-derived phase is very characteristic of the incorporation of the

polymer. Chitosan capping can modify the crystallization environment and provide abundant -OH and -NH₂ functionalities capable of interacting with inorganic nanophases [44,46].

Quantitative phase analysis also showed that there was significant rearrangement of the crystal phases in the presence of Co and chitosan. La₂O₃ was found to be the most prominent crystal phase in the composite (40.5 wt%), followed by Co(OH)₂ (20.1 wt%), chitosan L-ascorbate (17.5 wt%), AgCl (14.5 wt%), La(OH)₃ (5.0 wt%), and metallic Ag (2.4 wt%). The large reduction in the proportion of the AgCl phase from 88.7 wt% in Ag/La₂O₃ to 14.5 wt% in the composite cannot be taken to mean reduction in AgCl itself; rather, it shows the large introduction of the Co, La₂O₃, and chitosan crystal phases. In summary, the findings of XRD have strongly suggested the presence of the multiphase nanocomposite structure Ag/Co/La₂O₃/CS based on their clear diffraction patterns. The presence of metallic Ag/AgCl, La₂O₃/La(OH)₃, Co(OH)₂, and chitosan-derived crystallites would allow for synergistic interfacial properties that would be favorable for adsorption, catalytic, and antibacterial purposes. The complex structural findings obtained in the current study agree with other research studies which revealed the substantial influence of chitosan modification on the crystallinity of La₂O₃-based nanocomposites [44-46].

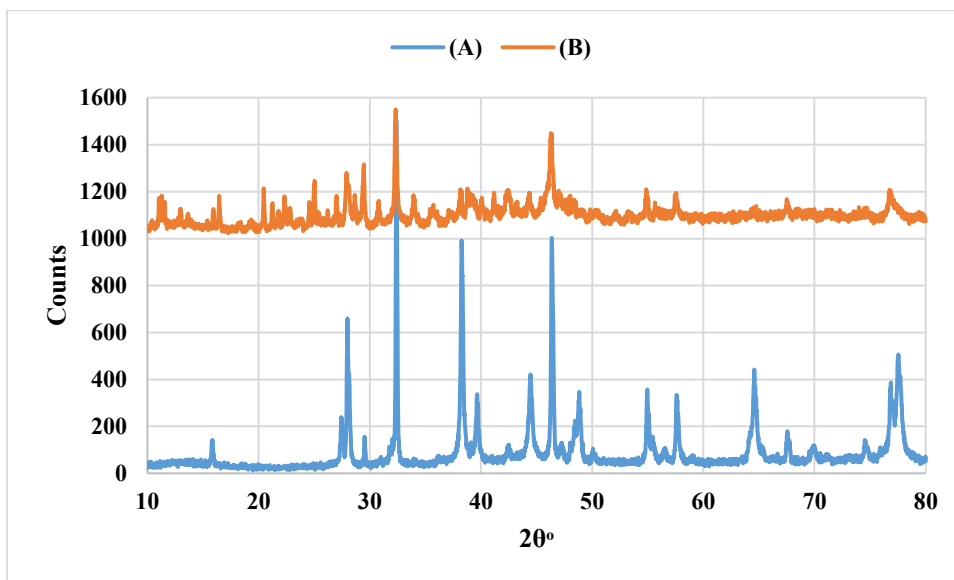


Figure 8. XRD patterns of (a) Ag/La₂O₃ NC and (b) Ag/Co/La₂O₃/CS NC.

3.3. Antibacterial activity

The antibacterial activity of rosemary extract, Ag/Co/La₂O₃ NC, and Ag/Co/La₂O₃/CS NC was measured using four Gram-negative and four Gram-positive bacteria through the agar well diffusion test, whereby the positive control was azithromycin (1 mg mL⁻¹) (Table S9 and Figure 9). The rosemary extract did not possess any antibacterial activity since the amount of phytochemicals contained in the extract was insufficient for the creation of the inhibition zones. On the contrary, the Ag/Co/La₂O₃ NC and Ag/Co/La₂O₃/CS NC were found to have broad-spectrum antibacterial activity, which means that the incorporation of Ag, Co, and La₂O₃ into

the hybrid nanocomposite highly increased the effectiveness of the antimicrobial agents. It should be mentioned that similar results were obtained during the investigation of antibacterial activity of lanthanum and cobalt nanomaterials when the role of synergism between metallic nanoparticles and metal oxides in the formation of inhibition zones was determined [17,47-50]. In the case of Gram-negative bacteria, the Ag/Co/La₂O₃ NC inhibited *S. typhimurium*, *K. pneumoniae*, and *E. cloacae* strains with inhibition zones of 13, 12, and 12 mm, respectively, whereas no zone of inhibition was found for *E. coli*. After chitosan functionalization, there were significant changes in the antibacterial properties of nanocomposites. The Ag/Co/La₂O₃/CS NC was active towards *E. coli* (12 mm), and the inhibition zones towards *K. pneumoniae* and *E. cloacae* increased to 14 and 16 mm, respectively. Surprisingly, the inhibitory effect on *S. typhimurium* vanished after chitosan modification. This may indicate that the polymer coating has influenced the accessibility of active surface sites and cell membrane interactions in a strain-specific way. Compared with azithromycin, the Ag/Co/La₂O₃/CS NC showed a better antibacterial effect against *K. pneumoniae* (14 mm versus 12 mm) and *E. cloacae* (16 mm versus 11 mm), while showing comparable efficacy against *S. typhimurium*. The results obtained clearly show that chitosan modification not only increases nanoparticle dispersion but also increases the interaction with the negative bacterial membrane due to electrostatic attraction of protonated amino groups (-NH₃⁺) of the polymer and the outer lipopolysaccharide layer of cells [17,48,50].

Furthermore, both nanocomposites had higher antimicrobial activity against Gram-positive bacteria due to the porous nature of peptidoglycan, making the penetration of nanomaterials easier. The Ag/Co/La₂O₃ NC had inhibition zones of 18, 21, 14, and 18 mm, respectively, against *B. subtilis*, *B. cereus*, *S. aureus*, and *S. epidermidis*. After modification with chitosan, the inhibition zone increased against *B. subtilis* (19 mm), *S. aureus* (16 mm), and particularly against *S. epidermidis* (22 mm), while it slightly decreased against *B. cereus* (19 mm). The highest value of inhibition zone obtained during these experiments was 22 mm against *S. epidermidis*, which exceeded the effect of azithromycin (19 mm). In comparison, activity against *B. subtilis* also exceeded the control sample (19 vs. 14 mm). In contrast, *B. cereus* remains highly sensitive to Ag/Co/La₂O₃ NC and azithromycin, with almost the same inhibition zone around 21 mm. Increased activity of the chitosan-modified nanocomposite is in accordance with numerous studies showing higher antibacterial efficiency of the chitosan-modified nanomaterials containing cobalt and lanthanum ions [17,47,18].

The antibacterial activity is related to the combined contribution of all nanocomposite constituents and not to the use of a single antimicrobial effect. The silver nanoparticles contribute to the presence of Ag⁺ ions, which can be attached to protein molecules of the cell membrane and thiol-containing enzymes, stop the respiratory chain, prevent DNA replication, and increase the permeability of the membrane. The mechanism of La₂O₃ consists of its adsorption on bacteria and induction of oxidative stress, while cobalt compounds facilitate the formation of ROS, leading to the oxidation of lipids, proteins, and nucleic acids. Moreover, chitosan can act through the positively charged amino groups, which will initiate electrostatic interaction of the bacterial envelope with the nanoparticles, resulting in their adherence and destabilization of the membrane

and introduction of Ag⁺ ions and ROS into the bacteria. In addition, chitosan is the stabilizing matrix preventing nanoparticles from aggregation and maintaining their highly active surface area. The combination of the effect of the destabilization of the membrane structure, oxidative stress, release of metal ions, inhibition of enzymatic processes, and damage of intracellular biomolecules accounts for the antibacterial activity of the Ag/Co/La₂O₃/CS nanocomposite described in the current research and other research on the antibacterial activity of the lanthanum-, cobalt-, and chitosan-based nanocomposites [17,47-50].

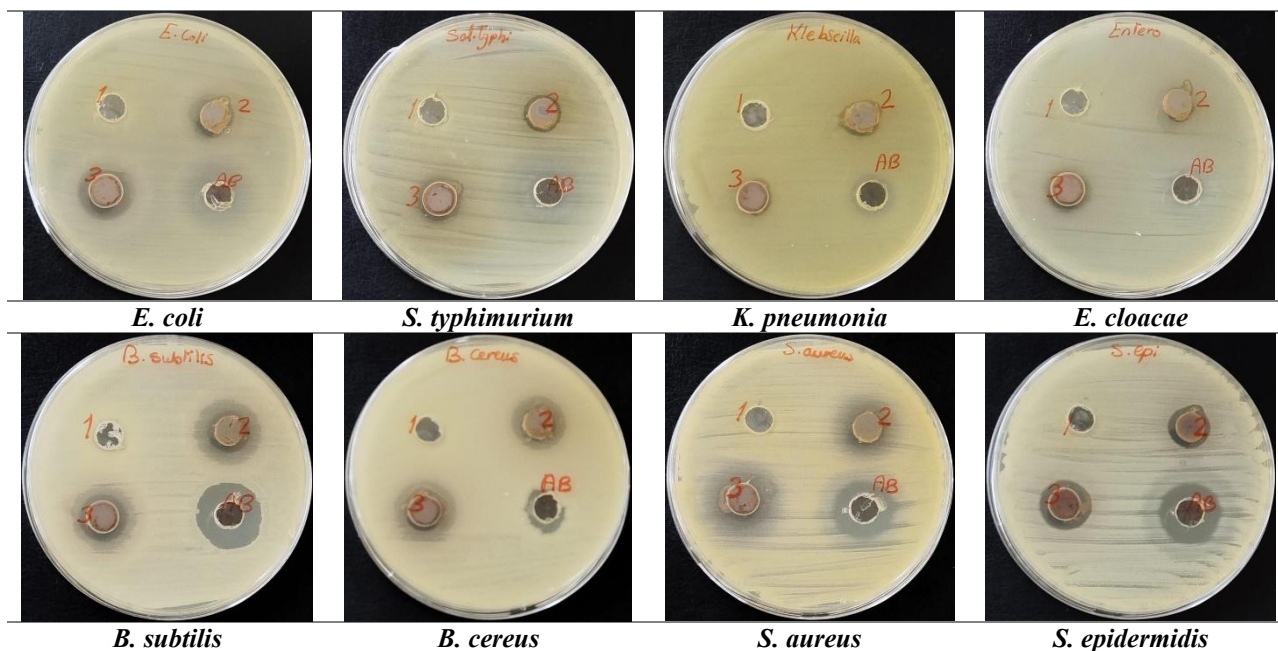


Figure 9. Representative agar well diffusion assay demonstrating the antibacterial activity of rosemary extract, Ag/Co/La₂O₃ nanocomposite (NC), Ag/Co/La₂O₃/chitosan (CS) nanocomposite, and azithromycin (1 mg mL⁻¹) against pathogenic bacterial strains. Wells are labeled as (A) rosemary extract, (B) Ag/Co/La₂O₃ NC, (C) Ag/Co/La₂O₃/CS NC, and (AB) azithromycin (positive control). Clear inhibition zones surrounding the nanocomposite-treated wells demonstrate the enhanced antibacterial activity of the synthesized materials, particularly after chitosan functionalization.

3.4. Photocatalytic Removal of MB and CV from Binary Solutions Using Ag/Co/La₂O₃/Chitosan Nanocomposite

3.4.1. Dark Adsorption of MB and CV onto Ag/Co/La₂O₃/Chitosan Nanocomposite

Dark adsorption was fast within the first 30 minutes and equilibrated after about 40 min (**Table S10**). After equilibration, dark adsorption of MB and CV was $33.5 \pm 1.4\%$ and $38.5 \pm 1.5\%$, respectively, where no significant increment occurred afterward. This implies greater interaction of CV with the Ag/Co/La₂O₃/CS surface, possibly via electrostatic and surface functional group interactions. However, since dark adsorption was considerably small compared to the irradiated dye removal, it cannot explain the process. Equilibration of dark adsorption before irradiation is still crucial since it will enable the concentration of dye molecules at active sites [51-53].

3.4.2. Control Experiments for MB and CV Removal

The control experiment revealed that photocatalysis played a prominent role (**Table S11**). Without catalyst and irradiation, MB and CV decontamination was insignificant (0.5 and 0.6%, correspondingly), while photolysis led to 4.5 and 4.0% removal. Without irradiation but in the dark, 33.5% MB and 38.5% CV decontamination were recorded, while in the presence of 0.50 g L⁻¹ Ag/Co/La₂O₃/CS catalyst, irradiation increased removal to 97.5 ± 0.7% and 94.0 ± 0.9%, respectively. It can be concluded that there is an appreciable synergy between the catalyst and irradiation. Such performance can be explained by the creation of charge carriers and then of reactive oxygen species on the semiconductor-solution interface [51-54].

3.4.3. Optimization of Photocatalytic Removal under Different Operating Conditions

3.4.3.1. Effect of Irradiation Time

Photocatalytic removal rate exhibited rapid increases with irradiation time, giving 85.5% and 78.0% MB and CV removal rates after 60 minutes and 95.0% and 88.0% after 120 minutes (**Table S12**). Maximum removal at 180 minutes was obtained as 97.5% and 94.0% for MB and CV removal, respectively. Progressive decreases in the removal rate with increasing irradiation time are explained by the exhaustion of easily removable dyes and competing intermediates [51,52].

3.4.3.2. Effect of Nanocomposite Dosage

A rise in the amount of catalyst concentration from 0.10 to 0.50 g L⁻¹ resulted in a rise in percentage dye degradation from 68.0 to 97.5% for methyl blue and 63.5 to 93.5% for Congo red dyes. Higher catalyst amounts provided a small improvement up to 98.1% and 95.7% at 1.00-1.50 g L⁻¹. The increase can be attributed to higher photon shielding/scattering due to catalyst aggregation at higher catalyst concentration [52]. Thus, 0.50 g L⁻¹ was selected as the practical optimum.

3.4.3.3. Effect of Initial Solution pH

Removal efficiency increased substantially from acidic to slightly alkaline pH, with peak values of 97.5% MB and 94.0% CV at pH 9.0. At pH 11, removal efficiency dropped to 92.5% and 90.5%, respectively. Improved efficiency in alkaline environment is probably due to favorable surface speciation, interaction between dyes and the surface, and increased presence of reactive species generated by hydroxyl radicals [51,53]. Reduced efficiency at very high alkalinity could be caused by surface charge alteration and scavenging effect. Therefore, pH 9.0 was chosen as optimum.

3.4.3.4. Effect of Initial Dye Concentration

An increase in the amount of binary dye concentration from 5/5 to 50/50 mg/L caused a reduction in MB and CV removal efficiency from 98.0 to 71.5% and from 97.0 to 68.5%, respectively. In cases where the pollutants concentration is increased, the limited number of active sites and photogenerated radicals may not be sufficient compared to the dye concentration, as well as higher dye absorbance of the photons [52,54]. The results therefore indicate that photocatalytic efficiency is strongly dependent on the pollutant-to-active-site ratio.

3.4.3.5. Effect of Temperature

The influence of temperature was relatively small. The MB removal was between 91.5 and 97.5%, and the CV removal was from 93.0% to 95.5% in the 20-60 °C temperature range. Lack of dependence on temperature suggests that this parameter was not the main restriction factor for the process within the studied conditions. Some increase in temperature may enhance diffusion and interphase mass transfer, but increased temperature may cause some opposite effects on adsorption or reaction equilibrium [52,53]. Importantly, the high removal obtained at 25 °C suggests no need for additional heat.

3.4.4. Photocatalytic Removal Kinetics of MB and CV

As evident from **Table S13**, the time-dependent removal of both the dyes was characterized by fast initial removal followed by slow removal rates gradually. The removal of MB increased from 42.0 to 85.5% during 10 to 60 min, followed by 97.5% after 180 min, while the removal of CV was 37.5 and 78.0%, and 94.0% at 10, 60, and 180 min, respectively. The respective experimental capacities of MB and CV were found to be 19.50 and 18.80 mg g⁻¹. The fast initial removal can be explained since large numbers of available sites and reactive species while the slow removal rate could be due to lack of readily reactive dye molecules and interference of intermediates [55-57].

From **Table S14** and **Fig. 10**, it is evident that the PSO model fitted very well with the experimental data, as evidenced by the higher R² values (0.9998 and 0.9996) compared to those obtained from the pseudo-first-order model (0.9923 and 0.9852), for MB and CV, respectively. Additionally, the predicted values (q_e) of 21.28 and 20.75 mg g⁻¹, using the PSO model, are quite comparable to the experimental values (19.50 and 18.80 mg g⁻¹). Despite this, the validity of PSO model does not prove conclusively the occurrence of chemisorption, especially when dealing with a coupled adsorption-photocatalysis process [55]. It should also be noted that recent works have pointed out that adsorption kinetic models are empirical descriptions and should be evaluated in conjunction with mechanistic information. Elovich model too offered a reasonable fit, especially for CV (R² = 0.9907), signifying heterogeneity of surface interactions with a decreasing rate of adsorption as the active sites get filled up gradually. On the other hand, the low values of R² from the intraparticle-diffusion model (0.8825 for MB and 0.9279 for CV), coupled with the large non-zero intercepts (C = 7.338 and 5.782 mg g⁻¹), suggest that intraparticle diffusion alone is not the only rate-limiting step, but external mass transfer, surface reaction, and diffusion in sequential order have played a role in the process [55,57]. Similarly, recent research findings confirm that non-zero intercepts in Weber-Morris's plot indicate boundary layer contributions. In conclusion, from the kinetic study it is evident that the removal of MB and CV by Ag/Co/La₂O₃/CS was efficient and fast at the same time. In addition, the faster removal of MB was achieved. It is worth mentioning that owing to good PSO results and weak dependence on the intraparticle-diffusion model, the multistep dye removal process is likely to involve surface processes, mass transport and photodegradation processes. However, owing to the decrease in the dye concentration being a decrease in its presence in the solution, a corresponding analysis should be performed.

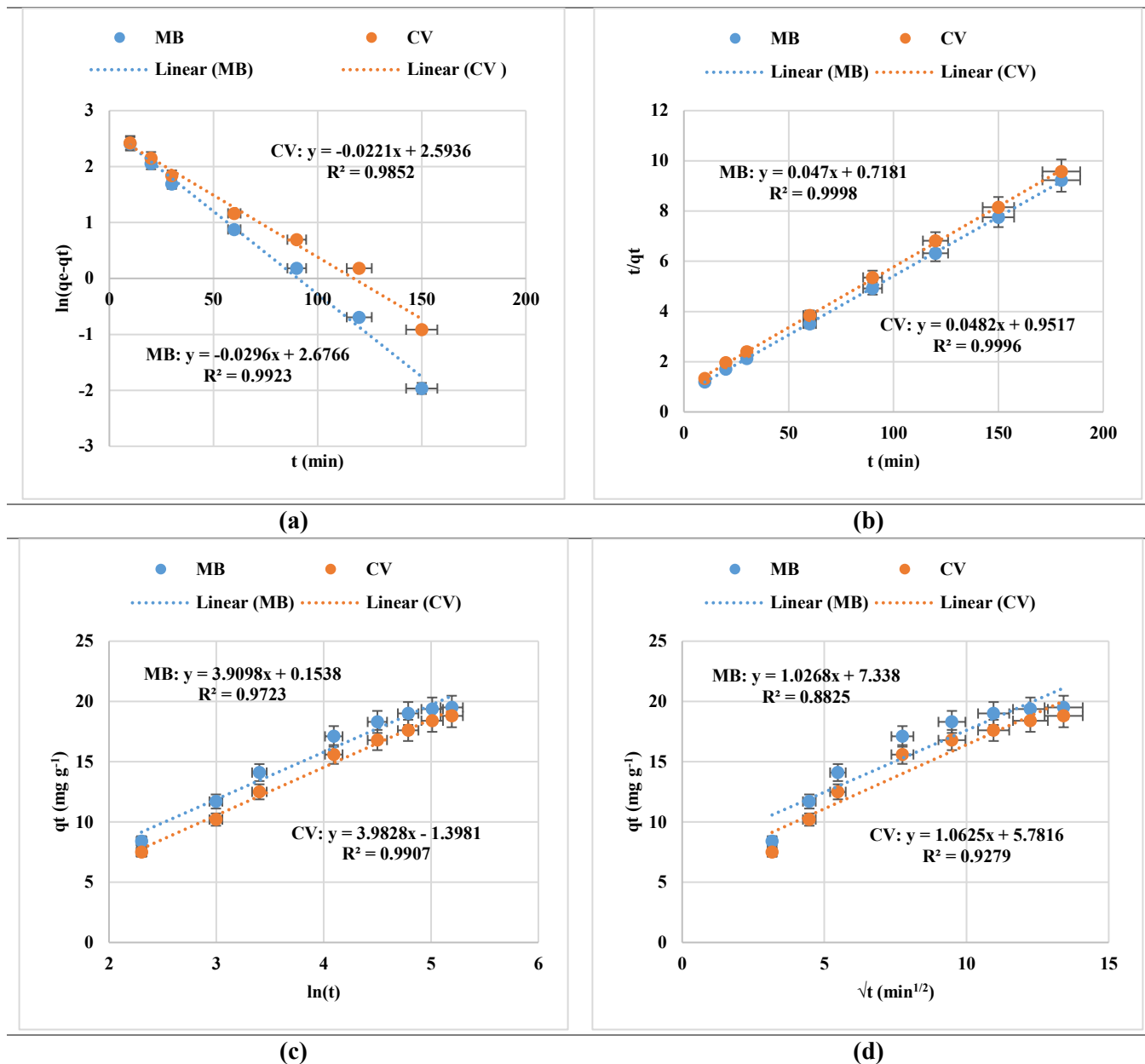


Figure 10. Linearized adsorption kinetic plots for the removal of methylene blue (MB) and crystal violet (CV) by the Ag/Co/La₂O₃/chitosan nanocomposite: (a) pseudo-first-order model, plotted as $\ln(q_e - q_t)$ versus contact time (t , min); (b) pseudo-second-order model, plotted as t/q_t versus contact time (t , min); (c) Elovich model, plotted as adsorption capacity (q_t , mg g⁻¹) versus $\ln(t)$; and (d) intraparticle diffusion model, plotted as adsorption capacity (q_t , mg g⁻¹) versus $\sqrt{t}$ (min^{1/2}) for MB and CV.

3.4.5. Adsorption Isotherms and Equilibrium Characteristics

According to the data presented in **Table S15**, the equilibrium values revealed an increase in the adsorption capacity with an increase in the initial concentration of dye. The obtained adsorption capacities were 71.50 and 68.50 mg/g at 50 mg L⁻¹ for MB and CV, respectively. This increase in capacity depends on the higher

concentration gradient and better site occupancy. Such results have been observed for recent nanocomposites for the removal of MB and CV [59,60]. According to **Table S16** and **Fig. 11**, Langmuir fitting proved to be highly effective for both MB ($R^2 = 0.9828$) and CV ($R^2 = 0.9867$), with maximum capacities of 75.925 and 75.062 mg g⁻¹, respectively. The relatively high KL values (0.765 and 0.522 L mg⁻¹) also reveal positive intermolecular interactions between dyes and the surface of the nanocomposite. The good Langmuir fit means that equilibrium data are well-described by a monolayer adsorption on a limited number of energetically similar sites; however, it cannot be seen as a confirmation of a perfectly homogeneous surface. In recent works concerning the MB/CV adsorption on the ZnO-graphene-based nanocomposite, the Langmuir model proved to be the most effective [59,61].

The Freundlich isotherm fit was also good, especially for CV ($R^2 = 0.9891$), with the values of n being 2.628 and 2.353 for MB and CV, respectively. Since ($n > 1$), the adsorption process is favorable, and hence there is significant heterogeneity on the surface. Both the Langmuir and Freundlich isotherms performing well simultaneously imply that there are heterogeneous sites present on the Ag/Co/La₂O₃/CS surface, but at the same time there is finite site adsorption. Similar fitting has been reported recently in other dye adsorption studies [60,62]. The Temkin correlation coefficient values were smaller (MB = 0.9741, CV = 0.9697), which demonstrates that the interaction between the adsorbate and adsorbent and the dependence of the adsorption energy on the surface coverage play a role in the equilibrium but do not explain all the properties of the system. The worst fit was obtained for the D-R model (especially for CV: $R^2 = 0.8082$); mean adsorption energies amounted to 3.193 kJ mol⁻¹ for MB and 2.784 kJ mol⁻¹ for CV. Both results are smaller than 8 kJ mol⁻¹ and can thus be considered indicative of physical adsorption based on the traditional D-R analysis [62,63]. However, the D-R energy values must be treated carefully, since they are calculated by means of the model and thus cannot prove the molecular mechanism of adsorption independently [61-63]. In general, from the above equilibrium results, there is high-capacity removal of both cationic dyes, with comparable Langmuir capacities (≈ 75 mg g⁻¹). The mixed Langmuir/Freundlich type of behavior suggests that finite site adsorption and surface heterogeneity influence the equilibrium process. Notably, since the above equilibrium measurements are for a system that has been irradiated for 180 minutes, the values of (q_e) will be representative of apparent overall uptake due to the adsorption and photocatalysis processes, rather than adsorption capacity alone. This distinction is essential because photocatalytic degradation continuously removes adsorbed and dissolved dye molecules, preventing interpretation of the Langmuir ($q_{\max}$) as a purely sorptive capacity.

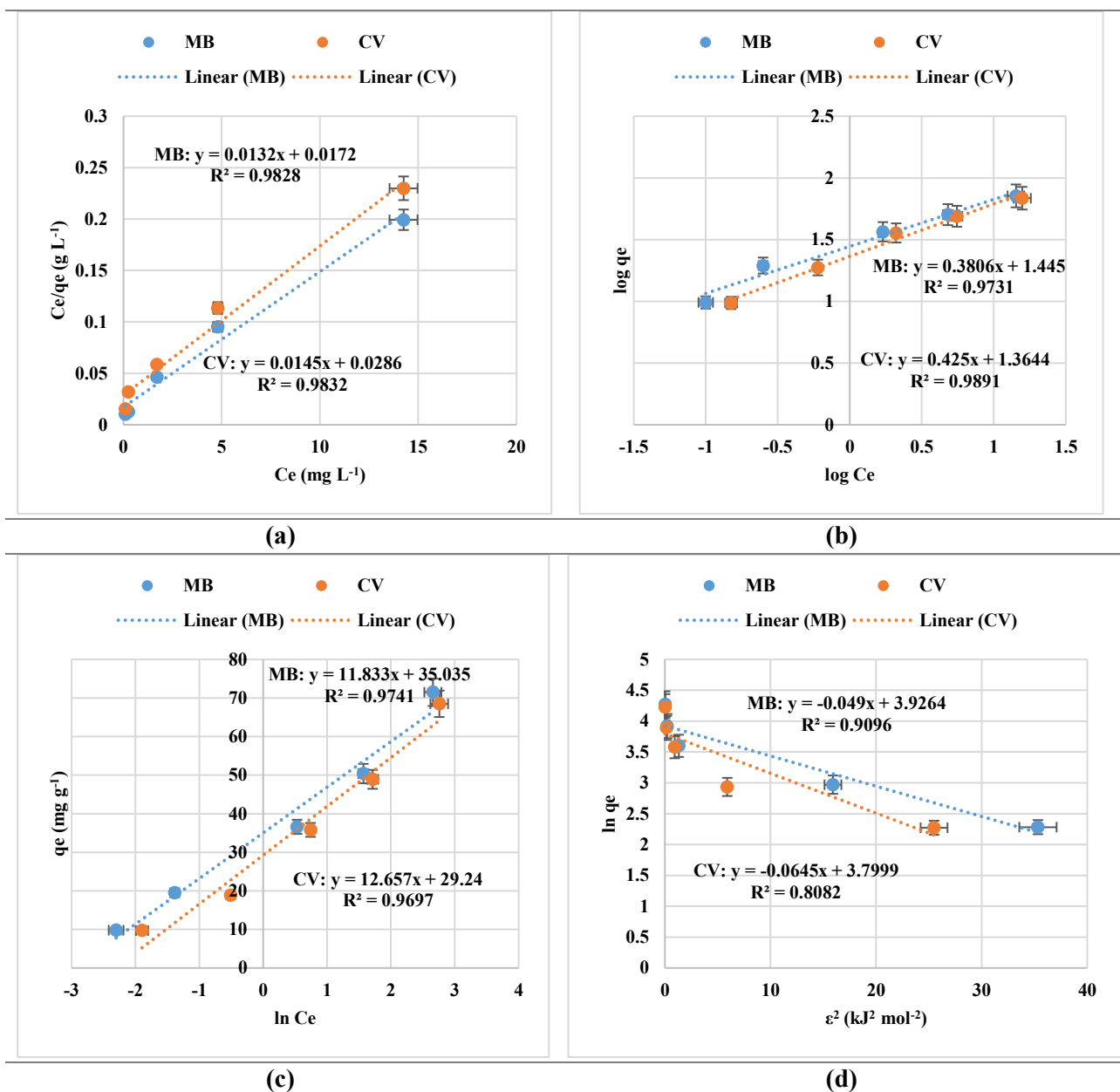


Figure 11. Linearized adsorption isotherm models describing the apparent equilibrium removal of methylene blue (MB) and crystal violet (CV) by the Ag/Co/La₂O₃/chitosan nanocomposite: (a) Langmuir isotherm, plotted as (C_e/q_e) versus (C_e) ; (b) Freundlich isotherm, plotted as $(\log q_e)$ versus $(\log C_e)$; (c) Temkin isotherm, plotted as (q_e) versus $(\ln C_e)$; and (d) Dubinin-Radushkevich (D-R) isotherm, plotted as $(\ln q_e)$ versus (ϵ^2) . Here, (C_e) is the equilibrium dye concentration (mg L⁻¹), (q_e) is the equilibrium adsorption capacity (mg g⁻¹), and (ϵ) is the Polanyi adsorption potential (kJ mol⁻¹). Linear regression was used to determine the corresponding isotherm constants and coefficients of determination (R^2) for MB and CV.

3.4.6. Thermodynamic Analysis of MB and CV Removal

The impact of temperature on the MB and CV degradation by Ag/Co/La₂O₃/chitosan nanocomposite was studied in the temperature range of 20-60 °C (Table S17). The efficiency of MB degradation rose from 91.5% at 20 °C to 97.5% at 60 °C, while for CV it increased from 93.0% to 95.5% at 50 °C, then slightly decreased

up to 94.0% at 60 °C. The distribution coefficient ($K_c = q_e/C_e$) also increased at higher temperatures. The thermodynamic data has been estimated based on Van't Hoff equation ($\ln K_c = -\Delta H^\circ/(RT) + \Delta S^\circ/R$) (**Table S18 and Table S19; Fig. 12**). Positive values of (ΔH°) were observed for both MB (+17.704 kJ mol⁻¹) and CV (+4.658 kJ mol⁻¹), implying that the whole removal process is endothermic in nature. The positive values of (ΔS°) for MB (+89.347 J mol⁻¹ K⁻¹) and CV (+44.251 J mol⁻¹ K⁻¹) may imply greater disorder at the interface during dye adsorption owing to the removal of water molecules at the adsorbent interface. Similar endothermic behavior has been reported for dye adsorption using chitosan-based nanocomposite adsorbents [64].

Values of (ΔG°) were negative for both dyes at all tested temperatures (**Table S20**), with values between -7.481 and -12.067 kJ mol⁻¹ for MB and -7.994 and -10.070 kJ mol⁻¹ for CV, thus confirming the thermodynamically favorable character of dye removal. The more negative (ΔG°) values with increasing temperature, especially in case of MB, also confirm the favorable influence of temperature on the removal process. On the other hand, the relatively small values of the squares of the correlation coefficient ($R^2 = 0.4082$ for MB and $R^2 = 0.2898$ for CV) of Van't Hoff plots point out that the graphs show deviations from linearity. Therefore, thermodynamic values determined should be considered apparent and can be used only as an indication of the temperature dependence of the process and its spontaneity.

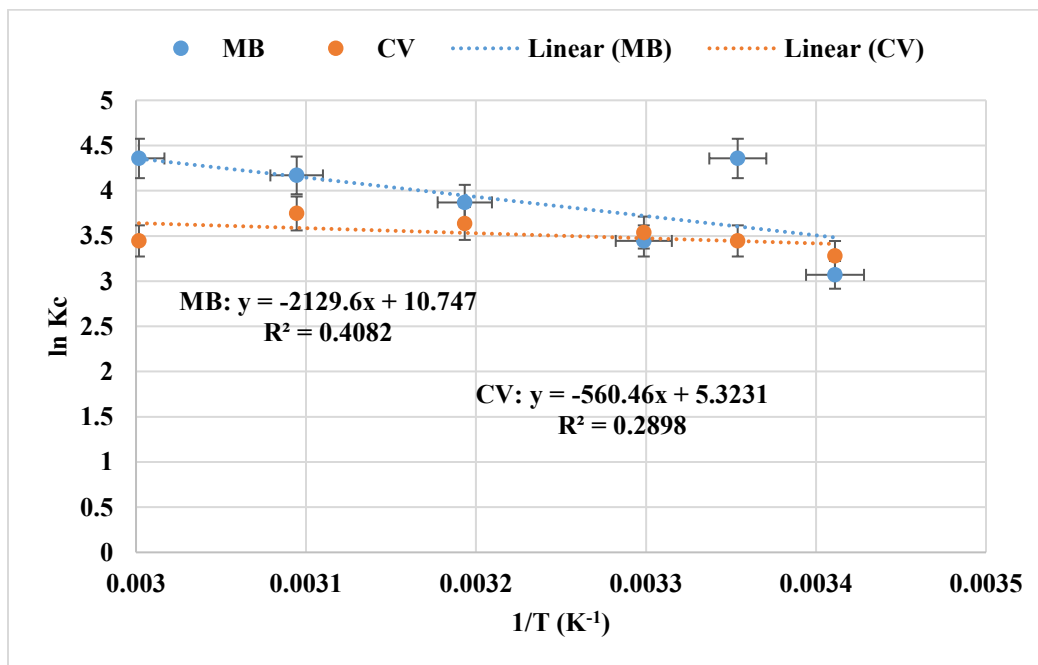


Figure 12. Van't Hoff plots for the thermodynamic analysis of MB and CV removal by the Ag/Co/La₂O₃/chitosan nanocomposite. The plots were constructed by linear regression of $\ln K_c$ versus $1/T$, where K_c is the adsorption equilibrium constant and T is the absolute temperature (K). The slope and intercept of each regression were used to determine the standard enthalpy change (ΔH°) and standard entropy change (ΔS°), respectively.

3.4.7. Reusability and Photocatalytic Stability of the Ag/Co/La₂O₃/Chitosan Nanocomposite

The ability to reuse the Ag/Co/La₂O₃/chitosan nanocomposite was investigated for seven successive photocatalytic cycles under the same experimental conditions (**Table S21**). Both dyes showed high removal efficiency with the nanocomposite, but there was a progressive decrease in their removal efficiency with an increase in the number of cycles. The MB dye removal efficiency dropped from $97.5 \pm 0.7\%$ at the first cycle to $91.5 \pm 1.3\%$ at the seventh cycle, implying an activity retention of 93.85%. Likewise, CV dye removal efficiency declined from $94.0 \pm 0.9\%$ to $82.8 \pm 1.5\%$, with 88.09% retention of activity. The relatively small loss in performance demonstrates good operational stability and reusability of the nanocomposite. This slight decrease in performance for CV can be related to the progressive saturation of the active centers due to the strong adsorption of dyes intermediates, incomplete regeneration or some variations in the available surface area due to multiple irradiations. However, the retention of more than 90% for the initial MB activity and around 88% for the initial CV activity after seven cycles suggests that the Ag/Co/La₂O₃/chitosan nanocomposite exhibits satisfactory stability for repeated photocatalytic use. The stability of the nanocomposite can be explained by its combined nature, where the chitosan matrix stabilizes the inorganic parts and allows their recovery after multiple treatments. Generally, these results demonstrate that the nanocomposite combines high photocatalytic efficiency with good reusability, which is an important consideration for practical dye-contaminated wastewater treatment.

3.4.8. Comparison of Photocatalytic Performance with Previously Reported Nanocomposites

Photocatalytic activity of the current Ag/Co/La₂O₃/chitosan (CS) nanocomposite was evaluated and compared with some recently developed photocatalytic nanocomposites for the removal of dyes (**Table S22**). Under the optimized reaction conditions, the current nanocomposite showed $97.5 \pm 0.7\%$ MB and $94.0 \pm 0.9\%$ CV degradation from an equimolar mixture (10 mg L^{-1} each) of the two dyes in 180 min at pH 9.0 and 25 °C with only 0.50 g L^{-1} of catalyst. The efficiencies obtained are equivalent to the efficiencies obtained by some recently synthesized semiconductor nanocomposites. For instance, the efficiency of approximately 99% MB degradation was observed for MgFe₂O₄-TiO₂-CS and TiO₂-ZnO-CS nanocomposites at optimized irradiation [65,66]. Other recent photocatalysts have also demonstrated high efficiencies toward MB and CV, including TiO₂-based materials and Co₂GeO₄/g-C₃N₄ heterojunctions [67,68].

Nevertheless, direct comparison of the results should be performed carefully since photocatalytic activity largely depends on the type of irradiation source, intensity of the light, amount of the catalyst, initial dye concentration, pH, reactor design, and duration of the treatment process [69]. The great benefit of this investigation is that MB and CV are treated simultaneously in a binary solution, thus competition occurs and, therefore, this model better reflects the real situation of wastewater containing various dyes. Also, the presence of rather high dark adsorption of MB (33.5%) and CV (38.5%) together with almost complete (97.5% and 94.0%, respectively) elimination in the presence of irradiation suggests the adsorption-photocatalysis coupling effect. Such multifunctional behavior is consistent with the increasing use of heterostructured and polymer-supported photocatalysts for simultaneous pollutant capture and degradation [69].

However, the Ag/Co/La₂O₃/CS nanocomposite also showed acceptable recycling efficiency since it maintained 93.85% of the initial MB degradation activity and 88.09% of the CV degradation activity in seven recycling cycles. Such efficient recyclable property is comparable with that of many of the recently reported heterostructure photocatalysts, for which recycling stability has been confirmed by several consecutive cycles [67,68]. The efficiency in the removal of dyes in the aqueous solution at room temperature, low amount of catalyst used, dual-dye degradation at once, and recyclability make Ag/Co/La₂O₃/CS a promising reusable material for dye-polluted water purification. However, such comparisons can only be regarded as performance-related, not necessarily quantitative, due to different conditions of experiments.

3.4.9. Silver and Cobalt Ion Release from the Nanocomposite

Ag and Co release behavior indicated that an increase in the absolute metal release was associated with an increase in the nanocomposite Ag/Co/La₂O₃/chitosan concentration (**Table S23**). At 0.10 g/L, the aqueous phase contained 0.18 ± 0.02 and 0.09 ± 0.01 mg/L of Ag and Co, respectively, and these values comprised 3.6% and 2.7% of the respective acid-extractable metal contents. The increase in the nanocomposite concentration to 1.00 g/L led to a corresponding increase in the aqueous concentrations of Ag and Co to 1.52 ± 0.10 and 0.73 ± 0.05 mg/L, respectively. Such an increase is likely explained by the fact that there was more total metal-containing material available at increased nanocomposite concentrations. At the same time, the percentage release followed an insignificant inverse trend, going down from 3.6 to 3.0% for Ag and 2.7 to 2.2% for Co. Thus, an increase in solid phase concentration did not lead to a proportional increase in metal mobilization.

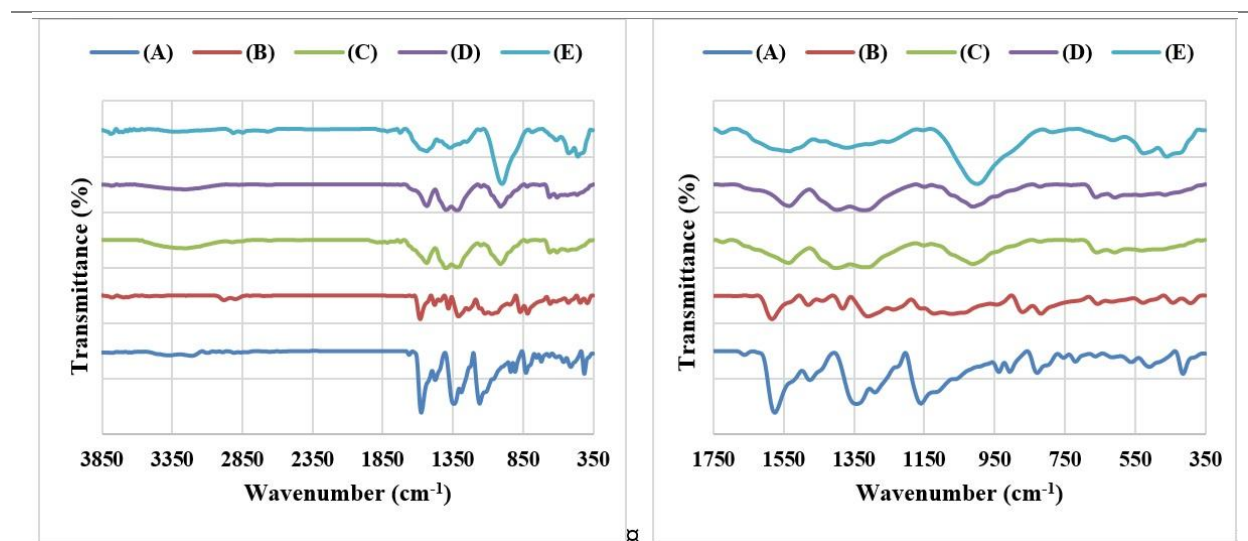
The small release percentage values further indicate efficient retention of Ag and Co within the matrix containing chitosan. Chitosan comprises amino and hydroxyl functional groups that can interact with the metal, and the polymer matrix could serve as a physical diffusion barrier, thus limiting the metal ion movement to the aqueous medium. Recent research has also shown that chitosan-based Ag nanocomposites have the potential to show controlled metal ion release due to the combination of metal coordination and diffusion through the chitosan matrix [70]. The somewhat larger release percentage of Ag than Co could be explained by differences in the access, localization on the surface, and chemical interactions of the two metals in the Ag/Co/La₂O₃/chitosan nanocomposite. It is important to note that the results obtained by the AAS analysis are related to the metal fraction within the aqueous phase and filter and cannot be considered as an indicator that the identified metals are present in the free form of Ag⁺ and Co²⁺ ions. Thus, the limited release of both metals indicates relatively strong retention of the metal components by the nanocomposite under the tested conditions, which is relevant when considering its environmental stability and applications requiring controlled metal release.

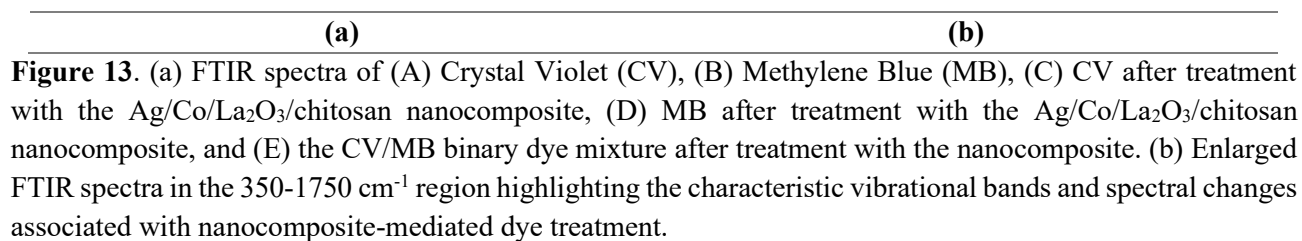
3.4.10. Reactive Oxygen Species Generation during Photocatalytic Treatment

The Ag/Co/La₂O₃/chitosan NC demonstrated an obvious concentration-dependent increase in ROS production, as proved by a gradual increase in the fluorescence of DCFH-DA (**Table S24**). At the lowest tested

concentration of $25 \mu\text{g mL}^{-1}$, the fluorescence was 118.4 ± 4.2 a.u. that corresponded to $18.4 \pm 1.5\%$ relative ROS production, while at the concentrations of 50, 100, 200, and $400 \mu\text{g mL}^{-1}$, the fluorescence gradually increased up to 143.7 ± 5.1 , 186.5 ± 6.3 , 254.8 ± 8.1 , and 341.6 ± 10.4 a.u., respectively. At the highest NC concentrations, the fluorescence values were 458.2 ± 13.7 and 501.5 ± 14.8 a.u. at 800 and $1000 \mu\text{g mL}^{-1}$, that corresponded to $132.6 \pm 4.0\%$ and $144.2 \pm 4.3\%$ relative ROS production, respectively. Therefore, an increase in NC concentration from 25 to $1000 \mu\text{g mL}^{-1}$ led to a 7.8-fold increase in the relative ROS production. A gradual increase in DCF fluorescence suggests an increase in oxidation activity of the nanocomposite at higher NC concentrations. Such concentration-dependent ROS production is consistent with the behavior reported for metal/metal-oxide nanocomposites, in which the coexistence of multiple redox-active components can facilitate oxidative processes and promote ROS formation [71].

The high ROS-producing ability of the Ag/Co/La₂O₃/chitosan NC may be explained by a synergistic effect of the Ag, Co-, and La₂O₃ particles, along with the high surface area of the nanostructured material and interfacial interactions. Redox-active metal centers are able to participate in electron transfer processes, thus contributing to the formation of reactive intermediates, whereas nanointerfaces may promote charge separation and oxygen activation. Furthermore, the chitosan matrix is also able to stabilize the nanoparticles and provide better dispersibility, thus providing additional reactive surface area. Similar multifunctional nanocomposites have been developed to produce a high amount of ROS using redox reactions and depleting the antioxidant defense systems of cells [26]. However, the DCFH-DA assay does not allow one to identify particular ROS species; it only indicates ROS presence. Thus, the results obtained using this method should be interpreted as a manifestation of ROS-generating potential depending on concentration and not the presence of specific ROS species, such as hydroxyl and superoxide radicals. Generally, the significant increase in ROS production at high concentrations of NC indicates that the oxidative stress may be responsible for the biological activity of Ag/Co/La₂O₃/chitosan NC.





3.4.11. FTIR-Based Assessment of Molecular Transformation of MB and CV

The FTIR spectra revealed significant molecular transformations in CV and MB under the influence of the Ag/Co/La₂O₃/chitosan nanocomposite (**Figure 13; Table S25**). Unprocessed CV was characterized by several characteristic bands at 3382, 3213, 1662, 1583, 1518, 1478, and 1440 cm⁻¹, while MB contained characteristic absorptions at 2987, 2902, 1640, 1587, 1526, 1483, and 1439 cm⁻¹. In the spectra of the treated dyes, there were significant simplifications, along with the attenuation or disappearance of many characteristic bands and shifts of the remaining signals. For CV, the disappearance of multiple fingerprint region bands along with the modifications in 3300-3261, 1665, 1532, and 1446 cm⁻¹, clearly indicates the modification of its molecular environment. In the same way, MB exhibited the disappearance of many original bands along with shifts in 3297, 1552, 1531, 1449, and 1407 cm⁻¹. Thus, FTIR band attenuation and shift can be considered as additional evidence of interactions between dye molecules and the surface of the nanocomposite, as well as molecular transformation due to treatment. Such FTIR band attenuation and displacement are commonly reported as complementary evidence of dye-nanomaterial interactions during nanocomposite-assisted dye removal [72].

In addition, the treated CV/MB binary mixture showed the highest spectral overview with the maintenance of a few bands at 3346, 2917, 1586, 1252, 1024, 990, 790, 612, 532, 464, and 415 cm⁻¹. In contrast to the more complex spectra of the untreated individual dyes, the loss of most vibrational bands associated with CV and MB implies significant modification and/or loss of dye molecules in the binary mixture. Therefore, the nanocomposite was effective in the presence of the binary dye mixture, which is especially valuable because binary systems have competitive interactions with the material's active centers [72]. Accordingly, the FTIR spectroscopy analysis indicates that there was some sort of interaction between the dyes and the nanocomposite and molecular transformations; however, the disappearance of FTIR bands cannot be considered the ultimate evidence of the mineralization of the dyes. However, considering the FTIR findings in combination with the results of the UV-Vis study on dye degradation, one can get additional proof of the effectiveness of Ag/Co/La₂O₃/chitosan for removing individual and binary dyes.

3.4.12. Proposed Mechanism of Simultaneous MB and CV Removal

The removal of both MB and CV simultaneously using the Ag/Co/La₂O₃/chitosan nanocomposite has been suggested to occur *via* the combined process of adsorption and photocatalysis, which is schematically shown in **Figure 14**. The first step involves adsorption of the two molecules on the surface of the nanocomposite due to interaction with amino (-NH₂) and hydroxyl (-OH) groups of chitosan, thus allowing their further reaction

on photoactive interfaces. On exposure to light, photon absorption in the Ag/Co/La₂O₃ leads to the formation of electrons and holes, whereas the proximity of the metal/metal-oxide interfaces may help separate these charges and inhibit their recombination [73,74]. Photogenerated electrons react with O₂ to produce superoxide radicals ($\bullet\text{O}_2^-$), while holes react with H₂O/OH⁻ to produce hydroxyl radicals ($\bullet\text{OH}$). The above-mentioned ROS, along with photo-induced holes, degrade the MB and CV molecules by cleaving and oxidizing their chromophores and aromatic units to produce intermediate products and eventually, in case of sufficient oxidation, low molecular weight compounds and mineralization. Experimental observation of concentration-dependent production of ROS adds further weight to the hypothesis of involvement of oxidative mechanisms; moreover, the dramatic reduction of characteristic FTIR bands suggests significant changes in molecular structure. Other similar photocatalytic chitosan-based systems have been shown to enhance dye degradation due to enhanced adsorption, interfacial charge transfer, and ROS generation [73]. Nanocomposite systems of Ag₂O/chitosan have already proven capable of producing $\bullet\text{O}_2^-$ and $\bullet\text{OH}$ radicals under visible light illumination for dye degradation via oxidative mechanisms [74]. Therefore, the proposed mechanism illustrated in **Fig. 14** reflects the sequence of processes: adsorption of the dyes → excitation → charge separation/transfer → ROS production → dye oxidation, and not just adsorption and photocatalysis.

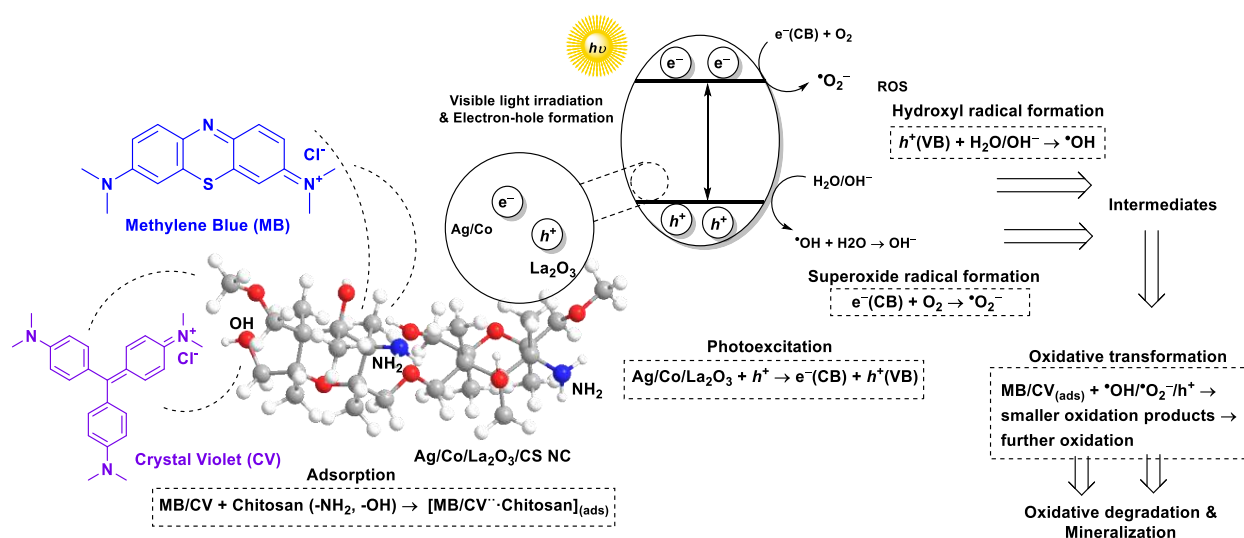


Figure 14. Schematic illustration of the proposed mechanism for the simultaneous removal of methylene blue (MB) and crystal violet (CV) by the Ag/Co/La₂O₃/chitosan nanocomposite.

4. Conclusion

An innovative multi-functional Ag/Co/La₂O₃/chitosan nanocomposite was fabricated using green synthesis approach using rosemary extract followed by chitosan functionalization. The nanocomposite revealed polycrystalline and multi-phased hybrid structure and was observed that chitosan greatly increases the dispersing ability, functionality and colloidal stability of nanoparticles. The band gap energy of the material was decreased from 3.89 to 2.94 eV because of the incorporation of chitosan and the zeta potential was

increased from +8.19 to +13.78 mV. The material exhibited broad-spectrum antibacterial activity and efficient ROS-generating capability.

Most importantly, the Ag/Co/La₂O₃/CS nanocomposite efficiently removed MB and CV simultaneously from binary aqueous solutions, achieving $97.5 \pm 0.7\%$ and $94.0 \pm 0.9\%$ removal, respectively, under mild conditions (0.50 g L⁻¹, pH 9, 25 °C, 180 min). The adsorption-photocatalysis coupling was supported by substantial dark adsorption, high irradiated removal, favorable kinetic behavior, and FTIR evidence of dye-surface interactions and molecular transformation. Langmuir apparent capacities were approximately 75 mg g⁻¹ for both dyes. Importantly, the catalyst retained 93.85% of its initial MB activity and 88.09% of its CV activity after seven cycles, while Ag and Co release remained relatively low. The new aspect of this work is the combination of the green synthesis by rosemary with multifunctionality of Ag/Co/La₂O₃ with surface functionalization by chitosan to produce a nanocomposite, which can simultaneously solve the problem of removal of binary dye and microbe pollution with generation of ROS and with low leaching of metals. Long-term stability, systematic identification, and toxicity characterization of the degradation products, complete speciation and fate of the released silver and cobalt, validation using actual wastewater samples, and flow-based experiments to determine requirements for regeneration and scalability should be the directions of future investigations. Generally, this study provides the proof of concept of the synergy of biosynthesis and biopolymer surface modification in developing multifunctional nanomaterials for integrated water purification and antimicrobial treatments.

Competing Interests

The authors declare that they have no competing interests.

Availability of data and materials

All relevant data are within the manuscript and available from the corresponding author upon request.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number NBU-FFR-2026-44-02

Funding

Not applicable

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