Cysteine-Coated Cadmium Sulfide Nanoparticles Conjugated with Curcumin for Antimicrobial Activity

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Nanoparticles have several applications in drug delivery. Attaching therapeutics to specially designed carriers enable precise delivery to specific cells. Nanostructures have unique physicochemical and biological features, such as an increased reactive surface area and the ability to pass through tissues and cell walls due to their small size, making them a promising material for biomedical applications. Cysteine-coated cadmium sulfide nanoparticles were prepared using a wet process at high pressure and temperature, followed by curcumin conjugation. The antioxidant, anticarcinogenic, and anti-inflammatory properties of curcumin are well acknowledged. Cadmium sulfide nanoparticles are of extremely good semiconducting material that shows fluorescence at a particular wavelength in spectrophotometric analysis. X-ray diffraction (XRD) of the nanocomposite was conducted to verify the crystalline nature of nanoparticles and to find the average crystallite size of cadmium sulfide nanoparticles. The Fourier-transform infrared spectroscopy (FTIR) confirmed the conjugation of cysteine with CdS and curcumin. Antibacterial activity of the synthesized material against Escherichia coli (E. coli) cells was assessed at different concentrations. The antibacterial activity of conjugated cadmium sulfide nanoparticles against E. coli bacteria was examined using the well diffusion method. The results showed that cadmium sulfide nanoparticles coated with cysteine and conjugated with curcumin had better cytotoxicity against bacterial infections caused by E. coli bacteria.

Keyword: Cadmium Sulfide Nanoparticles, Cysteine Coating, Curcumin Conjugation, Escherichia coli(E-Coli) Inhibition, Antibacterial Activity.

Introduction:

Nanotechnology refers to the manipulation and utilization of materials at the nano-scale (1–100 nm) [1], which has the potential to revolutionize many fields, particularly medicine. Nanoparticles are a prime example of this, finding numerous applications, especially in drug delivery systems. These nanoparticles, due to their small size and large surface area, can be engineered to carry therapeutic agents to specific tissues and cells within the body, improving the efficacy of treatments and minimizing side effects compared to traditional methods of drug administration [2][3]. The invention of scanning tunneling microscopes and the discovery of fullerenes and carbon nanotubes [4][5]. These breakthroughs paved the way for the exploration of semiconductor nanocrystals, including quantum dots, which have become a focus of interest for their unique optical and electronic properties.

One of the major advantages of using nanoparticles in drug delivery is their ability to be functionalized with targeting molecules, which allows them to deliver therapeutic agents specifically to diseased cells. These features significantly enhance the therapeutic index by ensuring that drugs reach their intended destination, such as tumors or inflamed tissues, while sparing healthy cells [6]. Nanoparticles, due to their small size, can also penetrate biological barriers, such as the blood-brain barrier, which has traditionally been a significant challenge for drug delivery in treating neurological diseases [7].

Nanomedicine, the application of nanotechnology for medical treatments, operates at the microscopic level to diagnose, treat, and prevent diseases. The ability to visualize, manipulate, and interact with biological systems at the molecular and cellular levels is a key advantage of nanomedicine. This includes the precise control over drug release profiles, where drugs encapsulated within nanoparticles can be released in a controlled and sustained manner, improving treatment outcomes [8]. In addition, nanocarriers are often designed to release their drug payload in a controlled manner, ensuring a steady therapeutic effect over time [9].

Cadmium sulfide (CdS) nanoparticles are particularly noteworthy due to their semiconductor properties. These nanoparticles exhibit strong fluorescence, a broad absorption spectrum, narrow emission bands, and excellent photostability [10]. Their optical properties make CdS nanoparticles highly suitable for biomedical applications, including bioimaging and photodynamic therapy. In addition, CdS nanoparticles are being explored for their antibacterial properties, as their ability to generate reactive oxygen species (ROS) under light exposure can lead to the disruption of bacterial cell membranes [11]. This makes them promising candidates for applications in antibacterial coatings and treatments, especially when combined with bioactive compounds like curcumin [12].

Curcumin, a compound derived from the turmeric plant (Curcuma longa), has garnered significant attention for its medicinal properties, including antioxidant, anti-inflammatory, and antimicrobial effects [13]. Chemically represented as $C_{21}H_{20}O_6$, curcumin is known for its ability to inhibit the growth, adhesion, invasion, and pro-inflammatory activity of a wide range of bacteria. This includes harmful pathogens such as Staphylococcus aureus, Escherichia coli, Streptococcus species, and Pseudomonas aeruginosa, which are commonly associated with wound infections. The effectiveness of curcumin against these pathogens makes it an attractive candidate for therapeutic applications. Researchers have successfully synthesized curcumin quantum dots, averaging 13.7 nm in size, which exhibit potent broad-spectrum antibacterial activity [14]. The quantum dots' small size enhances their ability to interact with bacterial cell walls and disrupt their function.

This research work demonstrates that cadmium sulfide nanoparticles (CdS NPs) coated with cysteine and conjugated with curcumin exhibit enhanced cytotoxicity against E. coli, showing potential as an effective antibacterial agent. Together, curcumin and cysteine-coated CdS nanoparticles may offer a powerful synergistic effect, enhancing the antibacterial action against pathogens like E. coli.

Objectives and significance:

The main objective of the study is to synthesize and characterize cysteine-coated cadmium sulfide nanoparticles conjugated with curcumin for antimicrobial analysis.
The nanocomposite synthesized will be beneficial in providing a variety of antimicrobial options for both industrial and medical use.

Experimental Analysis:

Synthesis of Cadmium Sulfide Nanoparticles:

The coprecipitation method has proven to be effective in producing CdS nanoparticles with controlled size and structure. The synthesized nanoparticles typically exhibit quantum size effects, with particle sizes ranging from 3-5 nm [15][16]. Firstly, 100 mL of a 0.01 M cadmium acetate solution was heated at 150°C for 10 minutes, and the pH was adjusted to 10 using a 1 M NaOH solution. Next, 100 mL of a 0.01 M sodium sulfide solution was added, and the mixture was heated with stirring for 15 hours. The resulting material was then centrifuged at 10,000 rpm for 20 minutes at room temperature, and the pellet was rinsed with distilled water. The material was centrifuged again at 10,000 rpm to obtain a solution containing CdS nanoparticles.

Coating of Cysteine to CdS Nanoparticles:

To coat CdS nanoparticles with cysteine, the pellet was dissolved in 50 mL of distilled water, and 20 mL of a 0.01 M cysteine solution was added. The sample was incubated at 100°C for 5 hours. Then, the sample was centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and dried. Ultrafine nanoparticles were found in the supernatant. Freeze the supernatant and then thaw it to allow ultrafine nanoparticles to settle at the bottom of the Falcon tube. This method allows the ultrafine quantum dots to sediment down more quickly without the use of ultra-high-speed centrifugation. The excess solution was removed, and the nanoparticles were dried at 45°C overnight. For the confirmation of cysteine coating on CdS NP's, a test tube was filled with a small number of prepared NPs and 0.5ml of 1 % Ninhydrin solution. The test tube was put into a hot water bath for five minutes, and the color of the solution was monitored.

Curcumin Conjugation to the Nanocomposite with the Help of the Glutaraldehyde Method:

The glutaraldehyde method is a widely used technique for crosslinking, particularly in biochemical and materials science applications, due to glutaraldehyde's ability to form covalent bonds with amine groups. In a 50 mL Falcon tube, 10 mg of CdS nanoparticles were mixed with 25 mL of a 0.01 M pyridine solution as a coupling buffer. The mixture was shaken firmly for 10 minutes. Glutaraldehyde was then added to the reaction vessel until a minimum concentration of 5% was achieved, with vigorous shaking. The mixture was incubated at 37°C for 3 hours at 100 rpm with shaking. Next, 5 mg of curcumin was added, and the blend was incubated at room temperature for 24 hours at 100 rpm with shaking. A sample of the mixture was collected as a pre-coupling reference. The solution was transferred to Eppendorf tubes and centrifuged at 7000 rpm for 10 minutes. Some of the supernatant was kept for post-coupling analysis, and the rest was discarded. In each Eppendorf tube, 1 mL of a 1 M glycine solution was added and incubated at 37°C for 30 minutes. After re-centrifuging at 7000 rpm, the supernatant was discarded. The particles in each tube were then washed with 1 mL of wash buffer containing 0.01 M Tris and 0.15 M NaCl. The pellets, as shown in Figure 1, were stored in Tris-Buffered Saline (TBS) at 4°C after a final centrifugation at 7000 rpm.

Figure 1 depicts the step-by-step procedure for the preparation of CdS quantum dots conjugated with curcumin.

Figure 1. Synthesis of CdS NPs coated with cysteine and conjugated with curcumin

Spectrophotometric analysis:

Spectrophotometric analysis of the nanocomposite was carried out to validate the optimum absorbance peak of the quantum dots and ensure optimum nanocomposite size.

FTIR:

FTIR analysis was carried out to ensure that all the molecules had bound successfully to the cadmium sulfide quantum dots, and a successful nanocomposite was synthesized. The resulting Graph is analyzed according to the reference graph of functional groups to ensure proper conjugation of the molecules to the nanoparticle.

XRD analysis:

XRD analysis was conducted of the nanocomposite to ensure that the proper 3D conformation of the nanocomposite was present to ensure its efficacy. The resulting XRD graph was analyzed under the reference JCPD card number provided in the literature.

Antimicrobial analysis:

Antimicrobial analysis of the nanocomposite was conducted by the well diffusion method against the coli strain to ensure that the nanocomposite carried potent antimicrobial activity at varied concentrations. Different concentrations of the nanocomposite were analyzed to deduce the efficacy and potency of the synthesized nanocomposite against the bacterial strain.

Results:

Spectrophotometric Investigation of CdS NPs Coated with Cysteine:

Cadmium sulfide nanoparticles coated with cysteine were analyzed spectrophotometrically at various wavelengths to examine the absorption ranges as shown in Figure 3, and to check the wavelength at which the particles release fluorescence.

CdS NPs produce green fluorescence, as shown in Figure 4. Observed NPs are ultrafine particles having maximum absorption at ~ 520nm, and also size of the NPs is relatively small.

X-ray Diffraction Analysis:

Cadmium Sulfide Coated with Cysteine NPs:

The X-ray diffraction analysis was performed to study the structural properties of the synthesized sample. The X-ray diffraction pattern is obtained for cysteine-coated CdS nanoparticles.

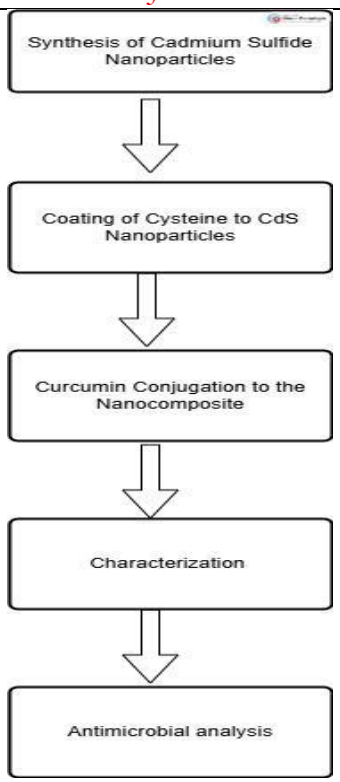

Figure 2. Flow chart of methodology

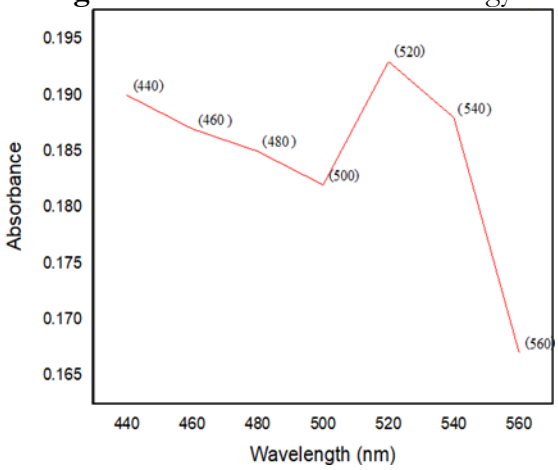

Figure 3. Absorbance spectra of Cadmium sulfide nanoparticles coated with cysteine

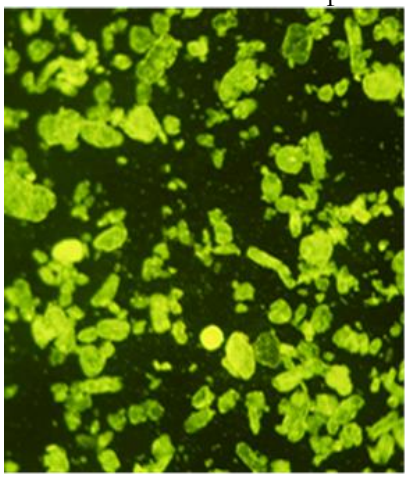

Figure 4. Cadmium sulfide nanoparticles coated with cysteine in white light

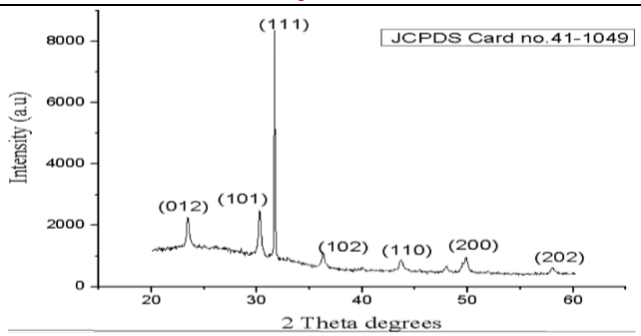

Figure 5. XRD patterns of CdS NPs coated with cysteine

The X-ray diffraction analysis was performed to study the structural properties of the synthesized sample. The Diffraction pattern is obtained for CdS nanoparticles coated with cysteine, shown in Figure 5. The synthesis of cysteine-coated CdS nanoparticles was confirmed by JCPDS card number (41-1049) as we got 2θ values at peak positions of 24.6°,30.31°, 32.6°, 36.32°, 43.71°, 49.80°and 58.5° that are indexed to the (012), (101), (111), (102), (110), (200) and (202) crystal planes and cadmium sulphide NPs coated with cysteine had hexagonal wurtzite phase.

By using the Scherrer formula Crystallite size was calculated.

$$D = \frac{K\lambda}{\beta\cos\theta}$$

Were

D=crystallite size,

K= shape factor=0.9,

λ= wavelength of x-rays=0.15406nm,

β= full width at half maximum,

θ= Bragg angle

The average crystallite size is about 18 nm.

Cadmium Sulfide Conjugated with Curcumin:

The X-ray diffraction analysis was performed to study the structural properties of the synthesized sample. The diffraction pattern is obtained for CdS nanoparticles coated with cysteine and conjugated with curcumin, shown in Figure 6. The peak at 23.1° with (012) crystal plane, which is in agreement with JCPDS card no of 9-816, confirms the presence of Curcumin. So, this peak showed that curcumin is conjugated to cadmium sulfide NPs coated with cysteine. Other peaks are positioned the same way as those of CdS nanoparticles coated with cysteine. The pattern showed dominant peaks of cysteine-coated CdS NPs conjugated with curcumin at positions of 2θ values of 23.1°,30.31°,32.6°, 36.32°, 43.71°, 49.8° and 58.5° that are indexed to the (012), (101), (111), (102), (110), (200) and (202) crystal planes matching with JCPDS card number of (41-1049). By using the Scherrer formula Crystallite size was calculated, and the average crystallite size is approximately 21nm.

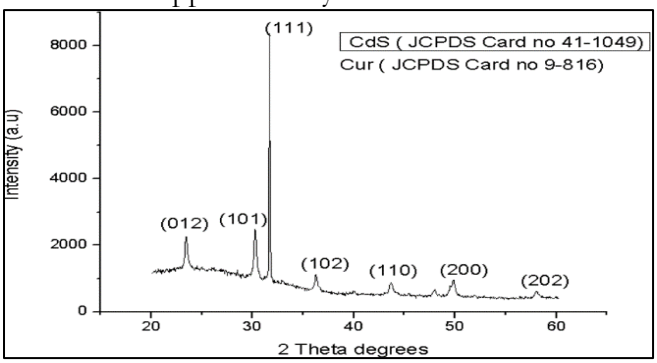

Figure 6. XRD patterns of CdS NPs coated with cysteine and conjugated with curcumin

The conjugation of cysteine-coated CdS nanoparticles with curcumin resulted in a significant XRD peak shift from 24.6° to 23.1°, indicating a lattice expansion due to surface interactions. Concurrently, the crystallite size increased from 18 nm to 21 nm, suggesting enhanced growth or aggregation of the nanoparticles following curcumin functionalization. These changes demonstrate the successful modification of CdS structure, potentially influencing its optical properties.

FTIR Analysis:

FTIR spectra of cadmium sulfide NPs coated with cysteine, shown in Figure 7, indicate that the C-H bond of cysteine is observed at a peak of 3604 cm^{-1}, N=H bond appears at 3330 cm^{-1}. C=O bond, C-C bond, and C-S bond appearing at 1400 cm^{-1}, 959 $cm^{-1,}$ and 720 $cm^{-1,}$ respectively, confirm that cysteine is coated on cadmium sulfide nanoparticles.

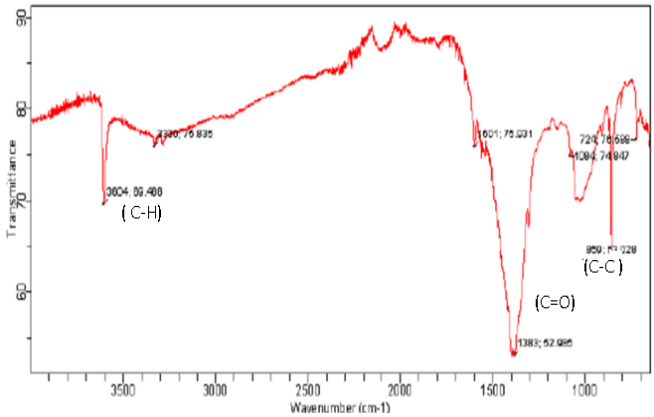

Figure 7. FTIR graph of Cadmium sulfide NPs coated with cysteine

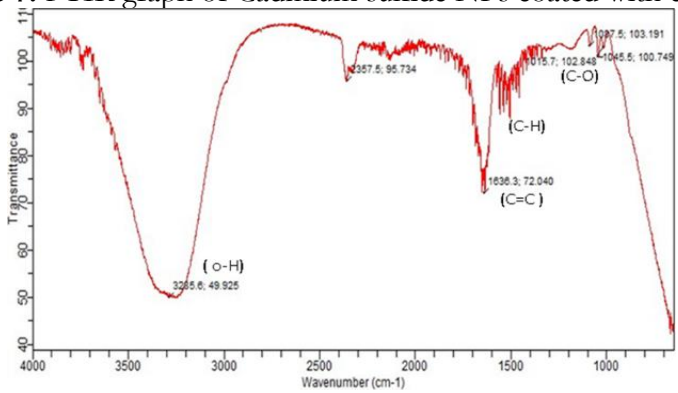

Figure 8. FTIR graph of CdS coated with cysteine and conjugated with curcumin

FTIR spectra of cadmium sulfide NPs coated with cysteine and conjugated with curcumin are shown in Figure 8. It indicates a broad absorption band at 3285cm^{-1} is due to O-H stretching vibrations. Stretching vibration of C=C is shown at 1636$cm^{-1,}$ and olefinic bending vibration of C-H bond due to benzene ring of curcumin is shown at 1500cm^{-1}, C-O bond present in curcumin is shown in the range of 1057cm^{-1} and 1045cm^{-1}.

Table 1. Standard table depicting the reference of different FTIR peaks with the functional group presence

Wavenumber (cm^{-1})	Peak Assignment
3650–3200	OH stretch
3100–3000	Aromatic CH stretch
3000–2850	Aliphatic CH stretch
1960–1680	C=O stretch
1650–1430	Benzene ring stretch
1450 ± 10, 1375 ± 5	Aliphatic CH_3 bend
1465 ± 20	Aliphatic CH_2 bend
1300–1000	C–O stretch
910–650	Aromatic CH bend

Antibacterial Activity of CdS NPs Conjugated with Curcumin:

Antibacterial Activity refers to the ability of a substance to inhibit the growth of or kill bacterial cells. This activity is critical in preventing and treating bacterial infections and is widely studied across various materials, including nanoparticles, natural compounds, and synthetic drugs. Two Petri plates were prepared and placed in a laminar flow hood for the well diffusion method. Luria Agar was poured onto the Petri plates and allowed to solidify. Then, 100 µL of E. coli bacterial culture was spread evenly on each plate using a spreader. Using a 1000 µL pipette with the tip's edge cut, four equal wells were created on each Petri plate.

On the first Petri plate (labeled E_1) as shown in Figure 9, 50 µL of cadmium sulfide nanoparticles coated with cysteine was added to the first well, 100 µL to the second well, 150 µL to the third well, and 200 µL to the fourth well. On the second Petri plate (labeled E_2), as shown in Figure 9, 50 µL of cadmium sulfide nanoparticles coated with cysteine and conjugated with curcumin was added to the first well, 100 µL to the second well, 150 µL to the third well, and 200 µL to the fourth well. The Petri plates were then incubated at 37°C for 48 hours.

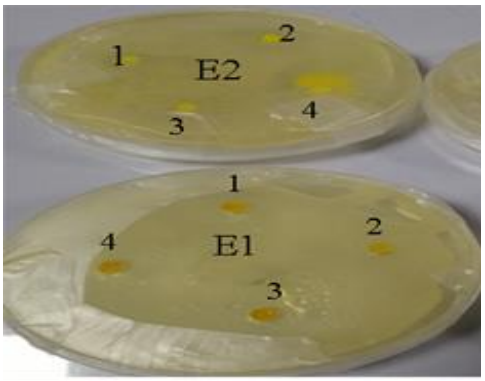

Figure 9. Petri plates containing the Zone of Inhibition

Measuring Zone of Inhibition:

Then, after 48 hours, the zone of inhibition was measured, and the following values were taken.

Values of zone of inhibition with E. coli bacterial culture and CdS NPs coated with cysteine and conjugated with curcumin are tabulated below.

Table 2 shows the values of the zone of inhibition of CdS NPs coated with cysteine and conjugated with curcumin with E. coli bacterial culture. As observed, increasing the concentration of cadmium sulfide nanoparticles coated with cysteine from 50 µL to 200 µL results in a corresponding increase in the zone of inhibition. Moreover, even greater values are noted when using cadmium sulfide nanoparticles coated with cysteine and conjugated with curcumin. Hence, CdS NPs coated with cysteine and conjugated with curcumin had better efficiency of killing E. coli bacteria. These results indicate that curcumin possesses strong antibacterial properties, and when it is conjugated with cadmium sulfide nanoparticles coated with cysteine, they provide a dual mechanism for effectively targeting and eliminating E. coli bacteria.

Table 2. Zone of inhibition of CdS NP's coated with cysteine conjugated with and without curcumin

Cadmium Sulphide with cysteine	Zone of inhibition	CdS with curcumin conjugated	Zone of inhibition
50µl	0.4	50µl	0.5cm
100 µl	0.5	100 µl	0.65cm
150µl	0.6	150µl	0.78cm
200µl	0.7	200µl	0.86cm

Discussion:

The successful synthesis of cadmium sulfide (CdS) nanoparticles coated with cysteine and conjugated with curcumin demonstrates a promising route toward developing multifunctional nanomaterials with enhanced antimicrobial properties. CdS nanoparticles were chosen due to their unique semiconductor characteristics and their potential to generate reactive oxygen species (ROS), contributing to antimicrobial activity [2][3]. Surface functionalization with cysteine, a biocompatible amino acid, not only improved the aqueous dispersibility and stability of the nanoparticles through its thiol and amine groups but also provided active sites for further conjugation. Curcumin, a well-known natural polyphenol with established antimicrobial, antioxidant, and anti-inflammatory properties, was conjugated to the cysteine-coated CdS nanoparticles to synergistically enhance the bioactivity of the final nanocomposite. The absorption maximum at ~520 nm and green fluorescence production are consistent with the quantum confinement effect in CdS nanoparticles. For bulk CdS, the band gap is around 2.42 eV, corresponding to an absorption edge around 512 nm. However, as particle size decreases to the nanoscale (ultrafine particles), quantum confinement leads to a blue shift in the absorption and emission spectra. The observation of green fluorescence suggests that these nanoparticles are indeed within the size quantization regime. Many studies on cysteine-capped CdS quantum dots (QDs) report similar optical properties, often with tunable emission wavelengths depending on the precise size and synthesis conditions. The calculated average crystallite size of 18 nm is within the typical range for CdS nanoparticles [17]. Numerous studies confirm the hexagonal wurtzite structure for cysteine-capped CdS NPs, with crystallite sizes varying depending on synthesis parameters, often in the range of 10-30 nm. The use of the Scherrer formula for crystallite size calculation is a standard practice in XRD analysis. The reported FTIR peaks are characteristic of cysteine. The presence of C-H, N-H (from the amine group), C=O (from the carboxyl group), and C-S (from the thiol group) vibrations strongly confirms the successful coating of cysteine on the CdS nanoparticles. The thiol (S-H) group of cysteine is known to have a strong affinity for CdS surfaces, often forming a stable bond that helps stabilize the nanoparticles and prevent aggregation. The C=O peak at 1400 cm^{1} is consistent with the symmetric stretching of the carboxylate group, which can deprotonate and interact with the nanoparticle surface [18]. FTIR is a standard technique used to confirm surface functionalization of nanoparticles, and these findings are in good agreement with published literature on cysteine-capped CdS nanoparticles. The results inferred in this study are consistent with the existing literature and show positive antimicrobial activity, as was hypothesized by the literature. The conjugated system was then evaluated for its antimicrobial efficacy against selected bacterial strains, with results indicating a significant improvement in inhibition zones compared to free curcumin or uncoated CdS nanoparticles. This enhanced activity can be attributed to the synergistic effect of ROS generation by CdS, increased cellular uptake due to cysteine coating, and the potent antimicrobial action of curcumin. The study highlights the potential of such hybrid nanostructures for applications in antimicrobial coatings, drug delivery, and wound healing.

Conclusion:

Cadmium sulfide nanoparticles coated with cysteine were synthesized using the coprecipitation method, followed by the conjugation with curcumin. The resulting CdS nanoparticles coated with cysteine exhibited green fluorescence under white light and showed maximum absorbance at a wavelength of 520 nm in spectrophotometric analysis. X-ray diffraction analysis confirmed that the average crystallite size of the prepared nanoparticles was approximately 18 nm, and they possessed a hexagonal wurtzite structure. FTIR analysis identified the functional groups present in the synthesized CdS nanoparticles coated with cysteine and conjugated with curcumin. The antibacterial activity of these nanoparticles was evaluated against Escherichia coli (E. coli) cells at various concentrations. The CdS

nanoparticles coated with cysteine and conjugated with curcumin demonstrated the greatest zone of inhibition. The results indicated that these nanoparticles could exhibit enhanced cytotoxicity against bacterial infections caused by E. coli, including pneumonia, urinary tract infections, meningitis, cellulitis, surgical wound infections, gastroenteritis, endocarditis, and osteomyelitis, as well as against fungal infections.

Conflict of Interest Statement:

The authors declare no conflicts of interest regarding the publication of this paper.

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