Role of CdSe quantum dots in the structure and antibacterial activity of chitosan/poly ε-caprolactone thin films

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ABSTRACT

Chitosan/poly(ε-caprolactone) (Ch/PCL) semi-natural polymeric blend containing gradient concentrations of CdSe quantum dots (QDs) dopant were synthesized via hot injection method. Synthesized samples containing different concentration of CdSe QDs were characterized by X-ray diffraction and FTIR absorption spectroscopy. FTIR experimental data of synthesized samples shows the maintenance of characteristic vibrational band with a marginal variation in both intensity and position related to the increase in dopant concentration. XRD patterns reveal amorphous nature of prepared virgin blend and blend samples that contain small amount of QDs. Samples with higher QDs concentration, namely (0.008, 0.016) wt% show appearance of crystalline bands related to the (1 1 1) reflection plane and in agreement with JCPDS card no. 19-0191. Scanning electron microscopy (SEM) indicates that morphology of synthesized bio-composites is critically affected by addition of CdSe QDs.

Antibacterial tests reveal different inhibition zone related to increasing concentration of CdSe QDs and type of bacteria under investigation. Evaluation of The activity index % were also studied.

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1. Introduction

Chitosan is a natural polymer classified as polysaccharide that composed of a random distribution of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine with a chemical formula \((\text{C}_n \text{H}_{2n+2} \text{O}_m \text{N})_n\) [1] which can be obtained from deacetylation process of chitin that considered as the second most abundant polysaccharide primarily extracted from exoskeleton of sea creatures [2–4]. Chitosan gained its cationic nature due to amino groups that grants biological activity at low pH values that results in a high capacity to interact with negatively charged compounds including proteins or anionic polysaccharides.

Polycaprolactone (PCL) is a hydrophobic synthetic semi-crystalline polyester with the chemical formula \((\text{C}_n \text{H}_{2n} \text{O}_n)_n\) that synthesized by ring opening polymerization of monomer \((\varepsilon\text{-caprolactone})\) via cationic, anionic and co-ordination catalysts or by free radical ring-opening polymerization of 2-methylene-1,3-dioxepane [5]. PCL usually characterized by slow degradation rate combined with high plasticity and ductility that can help counter balance the rapid degradation of natural polymers and increase the structural stability of the scaffolds obtained from their blends [6,7].

Blending of natural and synthetic polymers namely PCL with cellulose, starch and chitin may result in a new class of materials suitable with desired properties for bio-application as unique [8–12]. The hydrophobic character of PCL decreases the physico-chemical interactions with cells laying on its surface and their blends considered as a good candidate for the construction of 3D scaffolds results from slow degradation rate and its ability to maintain its morphology and mechanical properties after implanted that enhance mechanical properties of chitosan based scaffolds especially in the wet state [13]. PCL/Ch blends have been used as scaffold materials in the controlled release of drugs like Ofloxacin [14] and in nerve tissue reconstruction [15]. Ch/PCL ratio of 75/25 has higher hydrophilicity and better mechanical properties and cell adhesion and proliferation than PCL scaffolds.

Quantum dots (QDs) are spherical nano-sized crystals that may be formed of almost all semiconducting metals including CdSe, CdS, CdTe, PbS and ZnS while, alloys or any other metals may be used [16,17]. Cadmium selenide (CdSe) may be considered as an archetypal quantum dot with size range from 2 to 10 nm in diameter (10–50 atoms). Many types of quantum dot will emit light of specific frequencies if electricity or light is applied to them. These frequencies can be precisely tuned by changing the dot’s size, [18,19], giving rise to many applications. QDs were introduced to...
biological cell as alternative fluorescent probes in recent years. It uses in biological imaging, bio-sensing and intracellular detection and targeting, solar cells, quantum computing, transistors, LEDs and diode lasers [20]. Density function theory (DFT) is computational quantum mechanical method utilized as a part of physical science, material science to research the electronic structure (the ground state) of numerous body system, specifically particles, and atoms. It is a standout amongst the most well-known and effective quantum mechanical ways to deal with matter.

The present work aims to introduce a routine characterization for a novel semi-natural polymeric blend containing gradient concentration of CdSe QDs. FTIR, density functional theory (DFT) and XRD were employed to study the vibrational modes of both constituents of organic matrix and that with inorganic dopant. In addition, the antimicrobial tests were performed to study the effect of CdSe QDs on different gram-positive, gram-negative and fungi.

2. Experiment and method

2.1. Materials

Chitosan of molecular weight $6.0 \times 10^5$ [2-Amino-2-deoxy-(1-4)glucopyranan] with the chemical formula $(\text{C}_6\text{H}_{11}\text{NO})_n$ supplied by Aldrich Co. Poly ε-caprolactone of average molecular weight 45,000 with chemical formula $(\text{C}_6\text{H}_{10}\text{O}_2)_n$ supplied by Aldrich in pellets form. Acetic acid, ethanol and other solvents of high purity were supplied by Sham Lab. Co.

2.2. Sample preparation

CdSe QDs were synthesized via ordinary hot injection route previously reported [21,22]. In the synthesis route 0.8 mmol of CdO was added to about 20 mmol of stearic acid in a tri-neck flask with smooth heating (70–110 °C) in nitrogen atmosphere. The temperature was raised gradually to 180 °C after the formation of cadmium stearate (colorless solution). One mmol of Se metal powder and 3 mL of trioctylphosphine (TOP) were injected to the flask. Six equal amount of reaction mixture were collected every 15 min to permit the QDs formation and growth. The samples were instantaneously cooled and diluted with toluene to disperse CdSe particles growth. Obtained QDs were washed in methanol media and centrifuged.

Chitosan and poly ε-caprolactone were dissolved in 0.2 M aqueous acetic acid and glacial acetic acid respectively. Ch/PCL (75/25) poly blend was prepared using casting technique. Gradient concentration of the QDs were added to form thin film of desired concentration. Samples were kept in evacuated disserter until use. Table 1 lists the abbreviation and sample composition.

2.3. Physical measurements

Single beam (Nicolet iS10, USA) spectrophotometer was used to record the FTIR experiment data in the spectral range (4000–400)

| Sample | Chitosan wt% | PCL wt% | CdSe wt% |
|--------|-------------|---------|----------|
| CdSe0  | 75          | 25      | 0.000    |
| CdSe1  | 75          | 25      | 0.001    |
| CdSe2  | 75          | 25      | 0.002    |
| CdSe4  | 75          | 25      | 0.004    |
| CdSe8  | 75          | 25      | 0.008    |
| CdSe16 | 75          | 25      | 0.016    |

X-ray diffraction scans were obtained using PANalytical X’Pert PRO XRD system using Cu Kα target with secondary monochromator (where, $\lambda = 1.540 \AA$, the tube operated at 45 kV–40 mA (Holland), the Bragg’s angle ($\theta$) in the range of (5–80°). In this analysis, the peak locations ($\theta$) in X-ray diffraction spectra are used to identify the different crystalline structures in the pure and doped films.

The morphology of the films was characterized by scanning electron microscope using SEM Model Quanta 250 FEG (Field Emission Gun) with accelerating voltage 30 kV, magnification 14× up to 1,000,000 and resolution for Gun1n. Size and shape of QDs determined using HRTEM (JEOL-JEM-2100) with accelerating voltage 200 kV while UV/Vis. measurement was performed using JASCO V770 Spectrophotometer.

3. Results and discussions

3.1. Characterization of prepared QDs

Fig. 1 revels UV UV/Vis optical absorption spectra of prepared CdSe QDs combined with high resolution transmission electron micrographs (HRTEM). Obtained micrograph shows that synthesized material are of spherical shape with size ranging from 4 to 5 nm. UV/Vis. optical absorption spectrum was found to be in agreement with that reported for the same sample previously reported [21,22].

3.2. Fourier transform-infrared spectroscopy (FTIR)

FTIR absorption experimental data of prepared pristine polymeric matrices and their blend films shown in Fig. 2 reveals the maintenance of basic vibrational groups belong to the backbone matrices of both Ch and PCL. The absorption bands observed in the chitosan spectra at 2951, 2872 cm$^{-1}$ can be assigned to the stretching vibrations of CH$_3$ while bands at 1648, 1555 cm$^{-1}$ may be attributed to (–C=O) secondary amide and (–C=O) protonated amine stretching respectively. Other bands located at 1165, 1025 cm$^{-1}$ are assigned to (C=O–C) symmetric and (C=O–C) symmetric vibration respectively. The band at 3429, 1724, 1412 cm$^{-1}$ is assigned to (O–H) overlapped to the (N–H) stretching vibrations, (C=O) carbonyl stretching and (CH$_3$) symmetric deformation. Same observation with marginal variation in peak position and/or intensity may be observed for PCL films. Fig. 3 also, shows the FTIR absorption experimental data of the FTIR experimental data of the FTIR experiment in the spectral range (4000–400 cm$^{-1}$ and with resolution of 2 cm$^{-1}$ to study the vibrational mode of the specimens.

Fig. 1. Reveals UV UV/Vis optical absorption spectra of prepared CdSe QDs combined with high resolution transmission electron micrographs.
distinct changes in the blend spectrum indicating some type of interaction between individual blend constituents. Such interaction can be discussed using both experimental and theoretical approach discussed in the next sections. Fig. 3 shows FTIR spectra of prepared polymeric blend doped with different concentrations of the inorganic filler (CdSe QDs). Analysed data of synthesized samples can be shown in Table 2. An obvious change in intensity was observed with increasing CdSe dopant content. The peaks at 1025 and 1412 cm\(^{-1}\) appear sharp in blend and (CdSe1) then become broad and increase in the broadness with increase concentration of filler up to 0.008 wt% then disappear in concentration of 0.016. The intensity of the peaks at 1555 cm\(^{-1}\) and 1724 cm\(^{-1}\) decrease with increasing CdSe dopant content. FTIR spectra are used to investigate the incorporation of PCL into chitosan biopolymer matrices by showing the absorption band positions and the vibrational modes.

### 3.3. Density function theory (DFT)

Density Functional Theory (DFT) a theoretical approach is employed to identify mechanism of interaction between the polymer matrices and measure the degree of agreement with experimental data for complex interaction between both chitosan and Poly e-caprolactone.

All calculations were performed using Gaussian 03 programs [26] within the DFT framework. Polymeric blend of (Ch/PCL) was optimized using the Becke’s three parameter hybrid functional, (B3LYP) correlation functional was also employed with the electron core potential basis set WLANL2DZ [27,28].

Both experimental and theoretical FTIR spectrum of Ch/PCL binary blend and polymer blend with dopant QDs are perceived to be in agreement with the suggested interaction and complexity between samples constituents shown in both Fig. 4a:d.

#### 3.4. X-ray diffraction analysis

The X-ray diffraction analysis for both pure chitosan and pure PCL show a broad band corresponding to amorphous nature of chitosan and two characteristic peaks at angles 2\(\theta\) = 21.2° and 2\(\theta\) = 23.5°, corresponding to the (1 1 0) and (2 0 0) crystallographic planes of semi crystalline nature of PCL biopolymer as shown in Fig. 5. XRD patterns of the Chitosan/PCL blend show that the two characteristic peaks of PCL were disappeared indicates the miscibility among two biopolymers, which means that the incorporation of PCL did not significantly affect the amorphous structure of chitosan. From Fig. 6, we can observed that the XRD patterns of the Ch/PCL/CdSe QDs bio-composites still kept the amorphous structure of Ch/PCL blend until the concentration of (0.008, 0.016) wt% of CdSe nanoparticles, two characteristic peaks of CdSe appear in concentration (0.008) wt% and make overlapping to form one peak in concentration (0.016) wt% which means that CdSe nanoparticles affect significantly on the amorphous structure of the blend.

#### 3.5. Scanning electron microscope (SEM)

The SEM has been used to explore the surface morphology and structure of studied samples. Fig. 7(A, B) presents SEM the micro- graphs of pure chitosan and pure PCL which showed smooth appearance of the surface. The SEM of Ch/PCL blend as shown in Fig. 7(C), doesn’t show any new features.

Addition of CdSe, QDs, with different concentrations to the biopolymer blend, the surface of bio composites become rough and the grain size was formed and varied in their shape according to CdSe QDs concentration as shown in Fig. 6(D–H). It is clear that the grain size at high concentration (0.008, 0.016) of CdSe QDs takes a definite shape and the CdSe were dispersed well in the blend. That’s mean that the morphology of Ch/PCL/CdSe QDs bio-composites is critically affected by addition of CdSe QDs. This reveals compatibility between the SEM and X-ray response of the present Ch/PCL/CdSe system.

| Peak position \((\text{cm}^{-1})\) | Band assignment | References |
|----------------------------------|-----------------|------------|
| 3429 \((\text{O}–\text{H})\) overlapped to the \((\text{N}–\text{H})\) stretching vibrations | 23            |
| 2951 \((\text{CH}_2)\) asymmetric stretching | 23            |
| 2872 \((\text{CH}_2)\) symmetric stretching | 24            |
| 1724 \((\text{C}–\text{O})\) carbonyl stretching | 24            |
| 1648 \((\text{C}–\text{O})\) stretching | 23            |
| 1555 \((\text{C}–\text{O})\) stretching protonated amine | 25            |
| 1412 \((\text{CH}_2)\) symmetric deformation | 25            |
| 1165 \((\text{C}–\text{O}–\text{C})\) symmetric stretching | 24            |
| 1025 \((\text{C}–\text{O}–\text{C})\) a symmetric and \((\text{C}–\text{O}–\text{C})\) symmetric vibration | 25            |
3.6. **Antibacterial tests**

During the last decade significant interest has aroused in the research on synthesis of Cadmium selenide (CdSe) QDs for biological, biomedical and pharmaceutical applications due to their known antimicrobial properties. The Cadmium ion exhibits broad-spectrum biocidal activity towards many different bacteria, fungi, and viruses [29–31].

The antimicrobial activity of the synthesized CdSe QDs doped with different concentration in polymer blend were tested. Thus the antimicrobial activity of the compounds was evaluated against two gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) as well as against the pathogenic fungus *Candida albicans* (C. albicans). The samples were seeded in petri dishes containing agar.
media (Agar 20 g + Beef extract 3 g + peptone 5 g) and the petri dishes were incubated at 36 °C. The inhibition zones were recorded after 24 h of incubation and summarized in Table 3. Each treatment was replicated three times.

Thus the antimicrobial activity percent (%) was evaluated from the relation:

\[
\text{Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100
\]

Fig. 8 shows antibacterial test results of CdSe QDs doped in polymer blend at room temperatures (RT). It was found that the size of the inhibition zone was higher against Bacillus subtilis and Staphylococcus aureus at 0.001 wt% CdSe QDs. Antibacterial activities index were found against all test cultures (Staphylococcus aureus, Bacillus subtilis) to be 83.3 and 52.2 respectively. While the activity index for Escherichia coli and Pseudomonas aeruginosa were 50.0 and 82.6 respectively. Moreover, the activity index of Candida Fungi was 81.5 at concentration 0.002 wt% CdSe QDs.

*Table 3* concentration of CdSe QDs against the diameter of inhibition zone and the activity index %.

| No. | Compound  | E. coli (mg/ml) | Pseudomonas aeruginosa (mg/ml) | S. aureus (mg/ml) | Bacillus subtilis (mg/ml) | C. albicans (mg/ml) |
|-----|-----------|----------------|-------------------------------|------------------|--------------------------|-------------------|
|     |           | D (mm) | A (%) | D (mm) | A (%) | D (mm) | A (%) | D (mm) | A (%) | D (mm) | A (%) |
| 1   | Chitosan  | 17.0   | 65.4  | 21     | 91.3  | 22.0   | 91.7  | 13.0   | 56.5  | 18.0   | 66.7  |
| 2   | Blend     | NA     | –     | 5      | 21.7  | 7.00   | 29.2  | 4.00   | 17.4  | 2.00   | 7.40  |
| 3   | CdSe(1)   | 13.0   | 50.0  | 19     | 82.6  | 20.0   | 83.3  | 12.0   | 52.2  | 4.00   | 14.8  |
| 4   | CdSe(2)   | 9.00   | 34.6  | 13     | 56.5  | 9.0    | 37.5  | 7.0    | 30.4  | 22.0   | 81.5  |
| 5   | CdSe(4)   | 12.0   | 46.1  | 17     | 73.9  | 19.0   | 79.2  | 10.0   | 43.5  | 15.0   | 55.5  |
| 6   | CdSe(8)   | NA     | –     | NA     | –     | 5.00   | 20.8  | NA     | –     | 5.00   | 18.5  |
| 7   | CdSe(16)  | 7.00   | 26.9  | 9      | 39.1  | 12.0   | 50.0  | 8.00   | 34.8  | 3.00   | 11.1  |
| Ampicillin | 26.0   | 100   | 23.0  | 100    | 24.0  | 100    | 23.0  | –      | –     | 27     | 100   |
| Clotrimazole | NA     | –     | NA    | –      | NA    | –      | NA    | –      | –     | 27     | 100   |

D, Diameter of inhibition zone; A, Activity index and NA no observed action.
No antibacterial activity was found at 0.008 wt% CdSe QDs except for Staphylococcus aureus and Candida albicans they show a moderate index activity and small inhibition zone. Among all, Bacillus subtilis exhibited maximum susceptibility, while Pseudomonas aeruginosa was found to be least susceptible to CdSe QDs. Increasing the synthesizing concentration of CdSe QDs resulted in significant reduction of antibacterial activity of CdSe may be due to increase in particle size of CdSe QDs. The difference of the sizes of zone of inhibition between the CdSe QDs synthesized at different concentration could be correlated to the difference in nanoparticles diffusion tendency in cells due to the difference in their sizes producing different amount of reactive oxygen species (ROS). The size of inhibition zone was different according to the type of bacteria and the concentrations of CdSe QDs. Based on the results obtained from Fig. 8 and the diameter of inhibition zone for different bacteria, it can be concluded that the maximum inhibition activity happens for Staphylococcus aureus. Fig. 8 also, demonstrates the similar extended results for different concentrations of CdSe nanoparticles antibacterial activity and it can be seen that the same results obtained. The maximum diameter has happened for S. aureus. All numerical data can be tabulated as seen in Table 3.

### 4. Conclusion

Semi-natural biocomposite of Ch/PCL containing gradient concentrations of CdSe QDs were successfully synthesized and characterized through X-ray diffraction and FTIR absorption spectroscopy. DFT approach was employed to investigate the reaction mechanisms of both polymer blend and samples that doped with the QDs filling material. Both FTIR experimental and experimental data shows the maintenance of characteristic vibrational band with a marginal variation in both intensity and position related to the increase in dopant concentration. XRD patterns reveal amorphous nature of prepared virgin blend and blend samples that contain small amount of QDs. Samples with higher QDs concentration, namely CdSe8 and CdSe16 shows appearance of crystalline bands assigned to 111 reflection plane reported previously and in agreement with JCPDS card no. 19-0191. Scanning electron microscopy (SEM) indicates that morphology of synthesized bio-composites is critically affected by addition of CdSe QDs. Antibacterial tests reveals different inhibition zone related to increasing concentration of CdSe QDs and sort of bacterial strain under investigation. Evaluation of The activity index % were also studied.

| Sample | C. Albicans (mg/ml) (fungi) | Bacillus subtilis (mg/ml) (gram positive) | S. aureus (mg/ml) (gram positive) | Pseudomonas aeruginosa (mg/ml) (gram-negative) | E. coli (mg/ml) (gram-negative) |
|--------|---------------------------|------------------------------------------|-------------------------------|-----------------------------------------------|---------------------------------|
| Chitosan| 1                         | 2                                        | 3                             | 4                                             | 5                              |
| Blend  | 2                         | 3                                        | 4                             | 5                                             | 6                              |
| CdSe1  | 3                         | 4                                        | 5                             | 6                                             | 7                              |
| CdSe2  | 4                         | 5                                        | 6                             | 7                                             | 8                              |
| CdSe4  | 5                         | 6                                        | 7                             | 8                                             | 9                              |
| CdSe8  | 6                         | 7                                        | 8                             | 9                                             | 10                             |
| CdSe16 | 7                         | 8                                        | 9                             | 10                                            | 11                             |

Fig. 8. The inhibition zone vs. the concentration of CdSe QDs.

### References

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