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N-Acetyl Cysteine-Cadmium Telluride NAC-CdTe QDs for Detecting the Damaged DNA in Cancerous Diseases

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Abstract

In this study water-soluble N-Acetyl Cysteine Capped-Cadmium Telluride QDs (NAC/CdTe nanocrystals) using N-acetyl cysteine as a stabilizer were prepared to investigate the utility of quantum dots (QDs) in distinguishing damaged DNA, (extracted from blood samples of leukaemia patients), from intact DNA (extracted from blood samples of healthy individuals) to be used for biosensing application. Based on the optical characterization of the prepared QDs, the XRD results revealed the formation of the NAC-CdTe-QDs with a grain size of 7.1nm. Whereas, the SEM test showed that the spherical size of the NAC-CdTe-QDs lies within 11~33nm. NAC-CdTe-QDs have superior PL emission properties at of 550nm and UV-Vis absorption peak at 300nm. The energy gap measurement through PL and UV-Vis was found to be 2.2eV and 2.3 eV, respectively. The interaction between the synthesized QDs and the extracted genomic DNA (both cancer damaged DNA and healthy undamaged DNA) was analysed optically, and compared to the normal reference DNA. The results showed a shift in the maximum fluorescence emission intensities (observed at 540nm nm for a damaged sample and 535 for a reference cell). Based on the obtained fluorescence results, the present study reached the conclusion that the prepared core/shell QDs could be employed as probes for diagnosing genetically disrupted DNA that is associated with malignant diseases from healthy DNA.

Keywords: NAC- CdTe nanocrystals, Fluorescence, Nucleic acid.

N-Acetyl Cysteine-Cadmium Telluride quantum dots للكشف عن تلف الحمض النووي في الأمراض السرطانية

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الخلاصة

تم تحضير CdTe القابل للذوبان في الماء والمغطى بـ NAC لتطبيق المستشعر الحيوي. تم تصنيع CdTe

في وسط مائي (المعبر عنها بالبلورات النانوية NAC / CdTe) باستخدام N-acetyl cysteine كمثبت تم تحضيره للتحقيق في فائدة النقاط الكمومية (QDs) لإعداد جهاز استشعار حيوي بصري لديه القدرة على تمييز الحمض النووي التالف (المرتبط بشكل كبير بالأمراض الخبيثة) من الحمض النووي السليم (المستخرج من الأفراد الأصحاء) التي يمكن استخدامها لتطبيق الاستشعار البيولوجي. استنادًا إلى التوصيف البصري لـ QD المُعد ، كشفت نتائج XRD عن تكوين NAC-CdTe QDs بحجم حبة 7.1 نانومتر. بينما يقترح اختبار SEM أن الحجم الكروي لـ NAC-CdTe QDs يقع في حدود 11 ~ 33 نانومتر. تتميز NAC-CdTe-QDs بخصائص انبعاث PL متفوقة عند 550 نانومتر وذروة امتصاص للأشعة المرئية وفوق البنفسجية عند 300 نانومتر. وجد أن قياس فجوة الطاقة من خلال PL و UV - vis هو 2.2 eV و 2.3 eV على التوالي. تم تحليل التفاعل بين QDs المُصنَّعة والحمض النووي الجيني المستخرج (كل من الحمض النووي التالف للسرطان والحمض النووي الصحي غير التالف) بصريًا ، ومقارنته بالحمض النووي المرجعي الطبيعي، لوحظ تحول في شدة انبعاث التآلق القسوى (540 نانومتر لعينة تالفة و 535 نانومتر لخلية مرجعية). بناءً على نتائج التآلق التي تم الحصول عليها ، توصلت الدراسة الحالية إلى استنتاج مفاده أنه يمكن استخدام QDs الأساسية / القشرية المحضرة كمجسات لتشخيص الحمض النووي المعطل وراثيًا المرتبط بالأمراض الخبيثة.

1. Introduction

In the last few years, nanoparticle quantum dots (QDs) have been a major area of interest within the field of optical biosensors due to their distinctive electronic and optical characteristics, including narrow emission bands, an ongoing broad absorption band, excellent quantum yields, and increased photo-bleaching resistance compared to conventional organic materials[1, 2]. QDs are becoming key components of a growing area of research by exploring their utility many biomedical applications. These include manufacturing fluorescent probes to investigate of their use in cell visualization, genomic detection, and a variety of optical sensors to meet different biological and medical needs[3, 4]. QDs have recently been investigated for their usefulness as fluorescent probes for DNA analysis, which constitutes the genetic makeup of all living organisms[5, 6]. It was demonstrated that prepared QDs can distinguish between the different synthesized DNA sequences. These results were based on the analysis of optical properties that the interactions of QDs with different DNA nucleotides (Adenine (A), Guanine (G), Cytosine (C) and Thiamine (T)) give different fluorescent emission maximums as well as different intensities[7]. It was demonstrated that QD's optical properties are surface electronic quantum status dependent. These properties (called states of a surface) are attributed to QDs high surface area ratio to volume [8]. Such characteristics can improve or reduce the photo-generated charge carriers' transfer rate. Other characteristics, such as ageing effects, the intensity of brilliance and spectrum brands, optical absorption (photoluminescence excitation), and quantum efficiency also seem to be affected by the surface states of the QDs[9, 10].

In this paper, N-Acetyl Cysteine Capped-Cadmium Telluride QDs (NAC capped CdTe QDs) using N-Acetyl Cysteine as a stabilizer were prepared whose structures were water-soluble and biocompatible due to utilizing N-A L cysteine as a stabilizer. There are relatively few publications in the literature that discuss how QDs behave in connection to DNA sequence variation. This study concentrates on the synthesis of a biological sensor from NAC- CdTe nanocrystals as a quantitative fluorescent probe for the comparison between undamaged (from normal cells) and damaged DNA (from malignant cells).

2. Experimental

2.1-Equipment and instruments used for the preparation of NAC-CdTe Nanoparticles

Three necked flask was used for the chemical reaction: one neck for nitrogen gas input and the second for the gas output, and the third for a thermometer. Hot plate heater with magnetic stirrer was used for homogenizing the chemical mixture under controlled reaction temperature. The flow of argon gas through the reaction flask was to expel the oxygen and to prevent oxide formation.

Additionally, the following instruments were employed in this work:

X-ray diffractometer (Shimadzu 6000-XRD, Japan), in the Nanotechnology and Advanced Materials Research Centre at the University of Technology, was used for XRD analysis.

FTIR Spectrophotometer (Shimadzu, IR TENSOR27, Japan); FTIR measurements were taken in the spectrum range of 500–4000 cm^{-1} in the Department of Materials Engineering at the University of Technology.

Humareader HS Elisa Microtiter Plate Reader (United states) in the Department of Biological Science at the University of Baghdad, Iraq.

UV/Visible Scanning Spectrophotometer (Shimadzu UV-1800) and spectro-fluorophotometer (RF-6000 Japan) in the Department of Chemistry at the University of Baghdad, Iraq.

2.3- Materials

The following chemicals were used in this work. All were supplied by Areej Al-Furat Company: Tellurium (Te powder, 99.8% purity, molecular weight 127.60 g/mol), sodium borohydride (NaBH_4 powder, 99.8% purity, molecular weight 37.84 g/mol), cadmium chloride (CdCl_2 powder, 99.99% purity, molecular weight 183.32 g/mol), NAC ($\text{C}_5\text{H}_9\text{NO}_3\text{S}$ powder, $\geq 99\%$ purity, molecular weight 163.19 g/mol), sodium hydroxide (NaOH , 99.99% purity, molecular weight 39.9971 g/mol), and 2-propanol industrial.

For the test, DNA was extracted from persons with cancer (leukaemia) and healthy persons for comparison.

2.4- Synthesis of NAC-capped CdTe QDs

The preparation of NAC-CdTe included two solutions: The first solution (NaHTe solution) was prepared by liquefying 0.189 gm of sodium borohydride (NaBH_4) with 0.125 tellurium powder (Te) in 25 ml of distilled water. Then the solution was put into a three-necked round flask with magnetic stirring to homogenize the solution. After that, nitrogen was pumped in to replace the air. This step was followed by heating the mixture to 70°C for 30 minutes until its colour turned dark crimson. Ultimately, the prepared NaHTe solution was preserved under nitrogen at room temperature.

The second solution was prepared by adding up to 0.180gm of cadmium chloride (CdCl_2) and 0.275 of N-Acetyl Cysteine (NAC) in 200 ml distilled water. The pH of the mixture was adjusted to 12.0 by dropwise addition of 1.0 M NaOH solution with stirring for 1 hour at room temperature. NAC-capped CdTe nanoparticles were prepared as a colloid. The second solution was transferred into a three-necked flask, where nitrogen was added to replace air. Then, 1mL of NaHTe solution was added by syringe with stirring at room temperature. The mixture was then heated to 90°C for 30min. This step was followed by immediately cooling the heated mixture to room temperature. At the end of the synthesis, excessive NAC–Cd complexes were removed by adding isopropanol to the mixture; the CdTe core QDs were precipitated. The precipitate was re-dispersed in ultrapure water after being produced [10].

2.5- Application of NAC-capped CdTe QDs for the detection of Damaged DNA

The QDs were tested to analyze their ability to distinguish DNA from different biological samples. DNA samples were extracted from leukaemia patients and healthy volunteers. DNA extraction was performed according to the manufacturer instructions using ReliaPrep™ Blood gDNA Miniprep System (supplied by Promega Corporation, USA). The quality and the

concentration of the extracted DNA samples were measured by nandrop (Thermo Scientific NanoDrop, USA)[11].

The experimental procedure included two main steps: In the first: an optical assessment of the prepared QDs was performed. While the second step involved the optical assessment of the core/ shell (at different concentrations) with 3 μl of undamaged DNA mixture, which was carried out using ELISA test that gives the absorption intensity of the different tested sample combinations. The absorption intensity refers to the strength of the correlation between the DNA and the QDs. This step helps determine the optimal correlation between the quantum dots and the DNA for the next step. Also, samples of 3 μl of damaged DNA mixed with 100 μl of QDs were evaluated.

3. Results and Discussion

3.1 –XRD Test Analysis

The diffraction pattern of NAC-capped CdTe QDs is shown in Figure1. The broad peaks confirm the nanoscale nature [12]. The pattern exhibits three peaks at $2\theta = 25.45^\circ$, 43.65° and 50.42° , corresponding to planes 111, 220, and 311, respectively. The average particle size for NAC-capped CdTe QDs using Debye-Scherrer equation[13, 14] is shown in Table 1:

$$D = 0.89\lambda/\beta \cos\theta \quad (1)$$

Where: D represents the size of the quantum dot, λ refers to the wavelength of the X-ray source (CuK α with $\lambda=1.5406 \text{ \AA}$), β is the Full Width at Half Maximum (FWHM), θ symbolizes the diffraction angle. Also, the interplanar spacing using Bragg Law $d = \lambda / 2\sin \theta$ was determined.

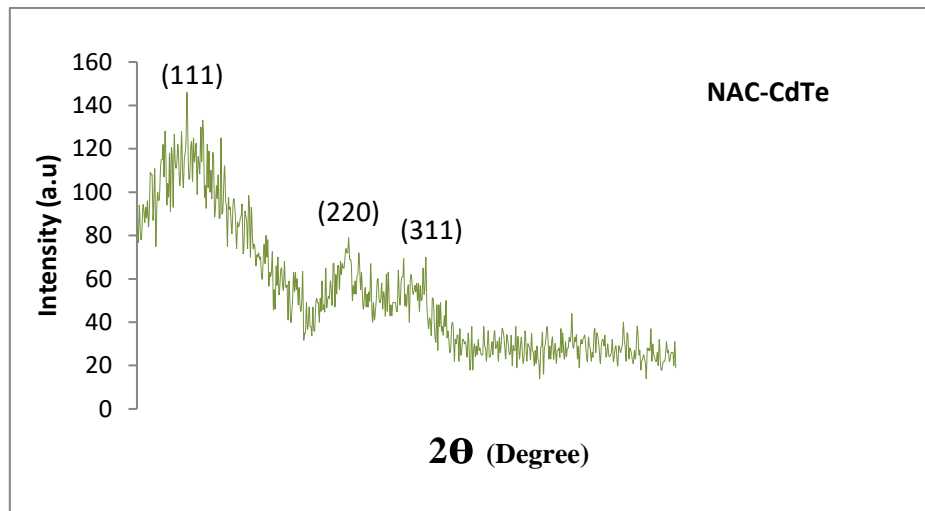


Figure 1: XRD pattern of the synthesized NAC-capped CdTe QDs.

Table 1: The grain size of the NAC-capped CdTe QDs

Sample	2θ (degree)	FWHM(degree)	dhkl Exp.(\AA)	Diameter Average (nm)	(hkl)
NAC/CdTe	25.45	6.8	3.4970426	1.198272	(111)
	43.65	1.2	2.0719637	7.1344582	(220)
	50.42	2.2	1.8084825	3.9927957	(311)

3.2- FESEM -EDX Analysis

Figure 2(a) illustrates a Field Emission Scanning Electron Microscopy(FESEM) photo of NAC-CdTe QDs nanoparticles. FESEM is a helpful instrument for evaluating the uniformity

of material covering broad areas. It provides a photographic appearance, the size and form of the grains, as well as the presence of faults or cavities of QDs nanoparticles. Based on that, the surface morphology of NAC-CdTe QDs nanoparticles showed a non-uniform particle size spreading on the surface. While the nanoparticles with spherical shapes appeared to be monodispersed with no agglomeration. Further analysis of the surface view revealed the presence of nanoparticles with circular shapes of sizes ranging between 11 to 33 nm. For precise measurements, these profiles were magnified with two different magnification powers, where the FESEM test showed an average size of NAC-CdTe nanoparticles within 200 nm.

Figure 2(b) shows a typical EDX spectrum verified in the 0–10 KeV binding energy region. The analysis confirms the formation of CdTe. The atomic ratio of the Cd functional group was relatively high compared to Te.

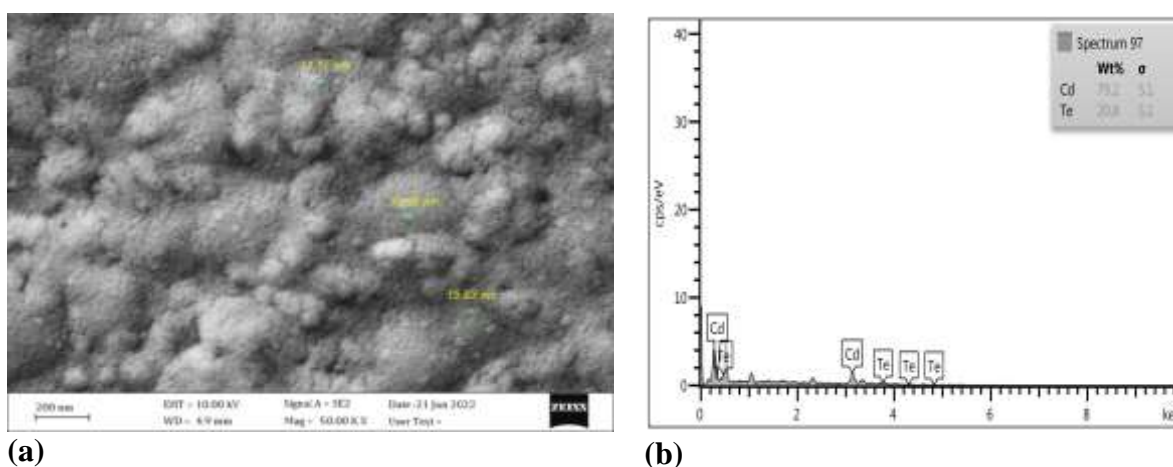


Figure 2: (a) FESEM image, and (b) Energy Dispersive X-Ray Spectroscopy (EDX) spectrum of the prepared NAC-CdTe thin films

3.3-FTIR

The FTIR test was used to confirm the conjunction of the capped NAC to the CdTe surface of the prepared quantum dots. The results of this analysis are depicted in Figure 3. The characteristic peaks of the NAC curve were located at 3373 cm^{-1} and 2550 cm^{-1} . They are caused by the asymmetric N-H and stretched S-H vibrations of the NAC, respectively. Additionally, peaks between 1500 and 1600, as well as at 1427 cm^{-1} , indicate the asymmetric stretching and bending vibrations of the carboxyl group. Furthermore, a comparison of the FTIR spectra of the NAC-capped CdTe QDs with those of free NAC revealed apparent differences [15]. In which the S-H bond's typical characteristic peak in the NAC-capped CdTe appeared to vanish; however, the other distinguishing peaks remained unchanged. This demonstrates that the NAC-capped CdTe lacked the S-H bond (the mercapto group). These findings indicate that the surface of the QDs had covalent bonds connecting the mercapto group of NAC with the cation of the QDs. Moreover, the S-H bond in the free NAC contributed to combining the QDs with DNA. These QDs exhibit exceptional water solubility and effective fluorescence stability, which are due to the NAC numerous carboxylate groups [16]. The synthesized nanocrystals water-solubility and biocompatibility are attributed to the polar carboxylic acid group of NAC on the out surface of NAC-CdTe, which are accessible to interact with various biomacromolecular including nucleic acids (e.g. DNA).

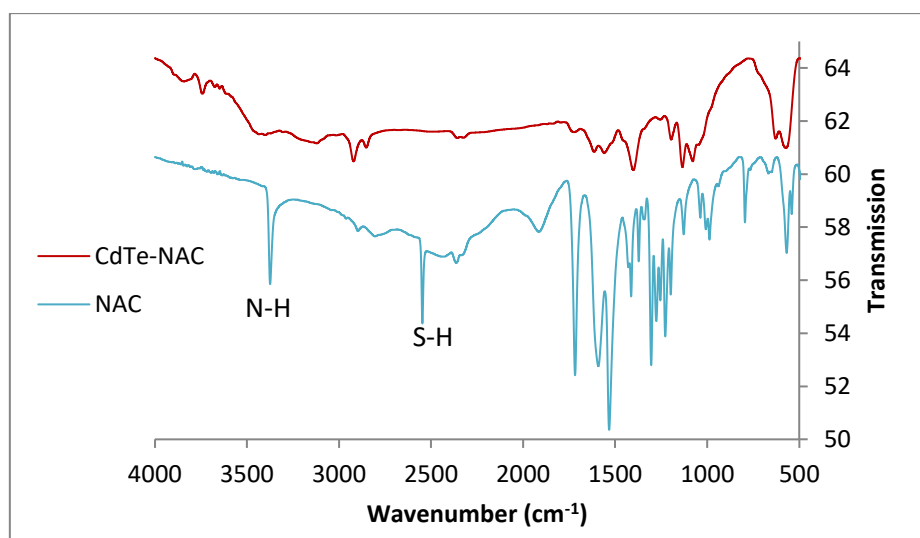


Figure 3: FTIR spectrum analysis of NAC-CdTe and the free NAC powders

3.4-Optical Properties

3.4.1- UV-Vis Absorbance

The absorbance spectrum of the NAC-CdTe QDs is demonstrated in Figure 4. The spectrum of the colloid is noted to have a peak at (300 nm).

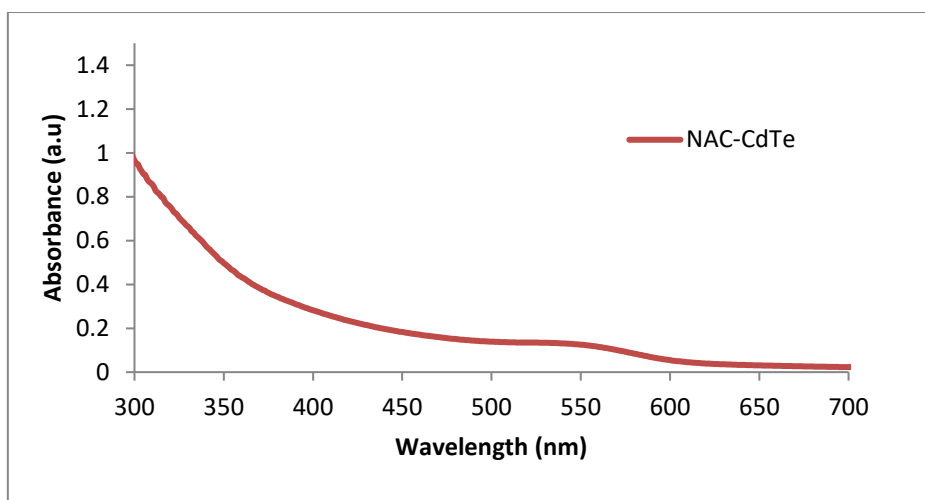


Figure 4: Absorption spectrum of NAC-capped CdTe QDs core-shell aqueous solution.

Energy band gaps can be calculated from the absorption Tauc's plot by drawing $(\alpha h\nu)^2$ versus $(h\nu)$ [17-18]. The following relation can be used to determine the optical direct band gap values:

$$\alpha h\nu = A(h\nu - E_g)^n \quad (2)$$

Where: α refers to the optical absorption coefficient, $h\nu$ is the photon energy, E_g is the optical band gap, n is a constant value which specifies the type of optical transition, for $n = 1/2$ and $3/2$ it is tolerable for direct transition, and when $n = 2$ and 3 , it is permitted for indirect transitions, and A is a constant called band tailing parameter[19-20].

The optical absorption coefficient α was calculated using the below equation:

$$\alpha = 2.303B/d \quad (3)$$

Where: B refers to the optical absorption readings and d donates the sample's thickness or path length through the quartz cuvette.

Figure 5 exhibits $(\alpha h\nu)^2$ versus $h\nu$ for NAC-capped CdTe QDs nanoparticles. The energy at which the extrapolation of the linear part of the curve crosses the x-axis is taken as the band gap energy for the NAC- CdTe QDs sample, which was at $E_g = 2.3$ eV; this coincides with the finding presented by Ebrahim et al. [21].

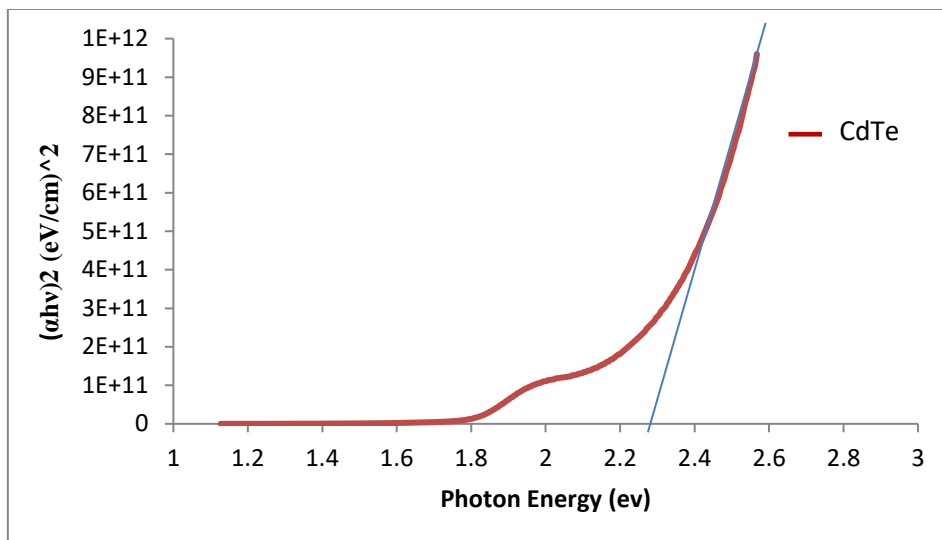


Figure 5: $(\alpha h\nu)^2$ versus photon energy for NAC-capped CdTe QDs nanoparticle.

3.4.2- The Photoluminescence (PL) Spectrum

The concentration of emitting species, the states relative energies (ground state and excited state), and electronic transitions were precisely examined by the Photoluminescence spectrum (PL). The PL spectrum of the colloidal of NAC-capped CdTe QDs nanoparticle (Figure 6) showed a broad band between 487 to 617 nm with a peak located at about 550 nm. Energy gap can be calculated from the relation ($E_g = 1240/\lambda$), λ represents the peak of emission in the PL spectrum [22]. The energy gap obtained in this case for NAC- CdTe QDs was 2.16 eV. This result is consistency with that of Carvalho et al.[23].

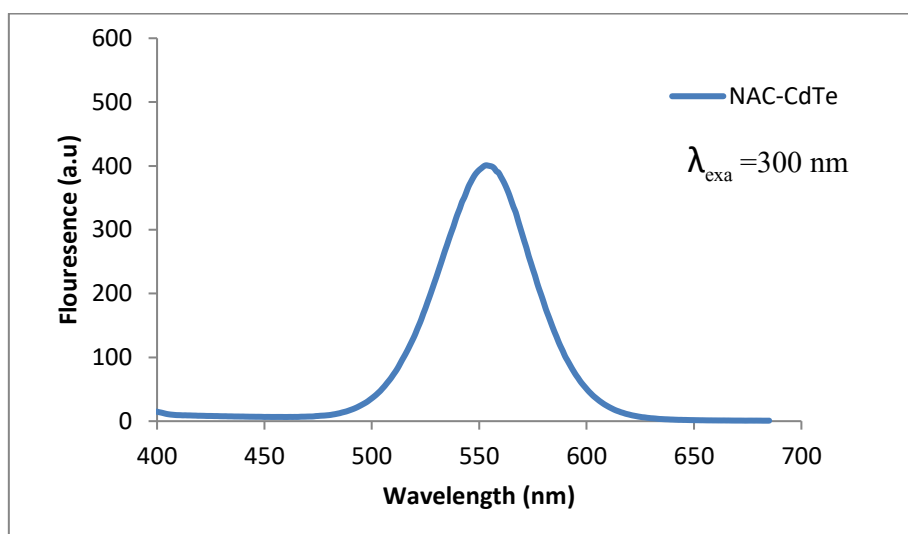


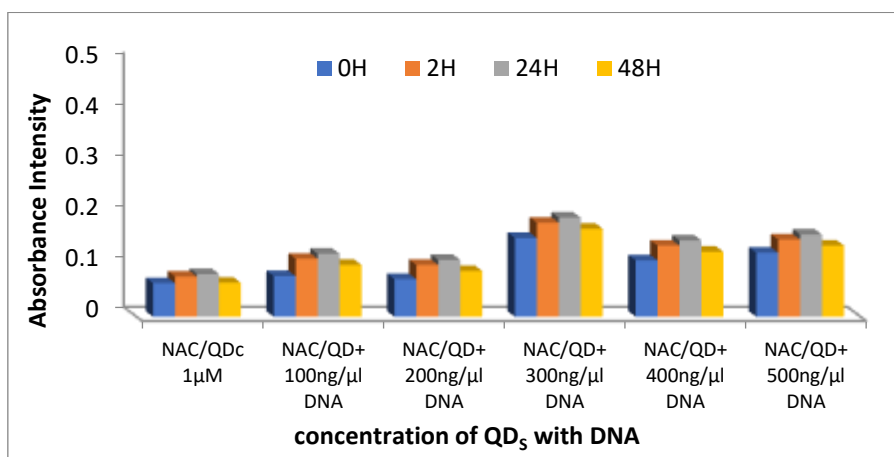
Figure 6: The photoluminescence (PL) spectrum of the colloidal of NAC-capped CdTe QDs nanoparticle

3.5-Optical Properties of QD Conjugated with DNA:

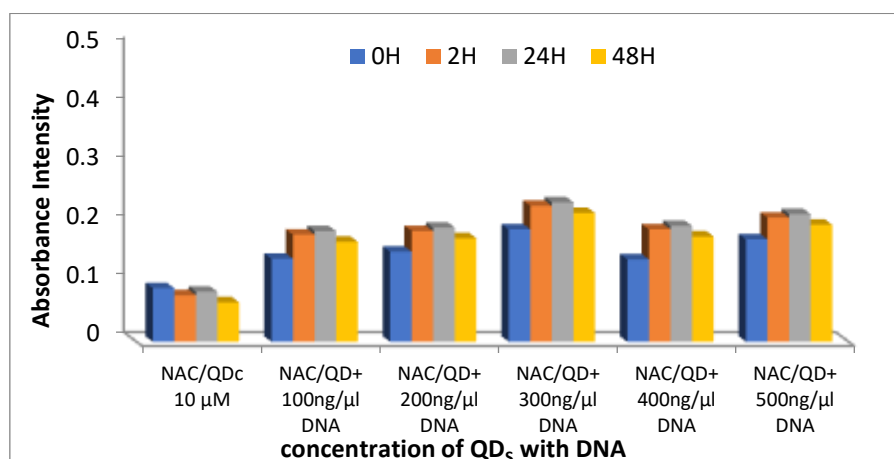
3.5.1- Absorbance Intensity of QDs Conjugated with the Reference DNA:

The absorbance test was carried out for samples prepared by mixing 3 μ l of healthy DNA with 100 μ l of NAC-capped CdTe of different concentrations (1 μ M, 10 μ M and 25 μ M) which were then placed in 96-well plates to determine the best concentration of QDs to conjugate with DNA, as shown in Figure 7(a, b, c). The plate was then inserted in a fully automatic ELISA reader to assess the absorption spectrum for the mixture and measure the absorbance intensity using a spectrophotometry at wavelength 492 nm which is close to the energy gap calculated for the quantum dots used in the research.

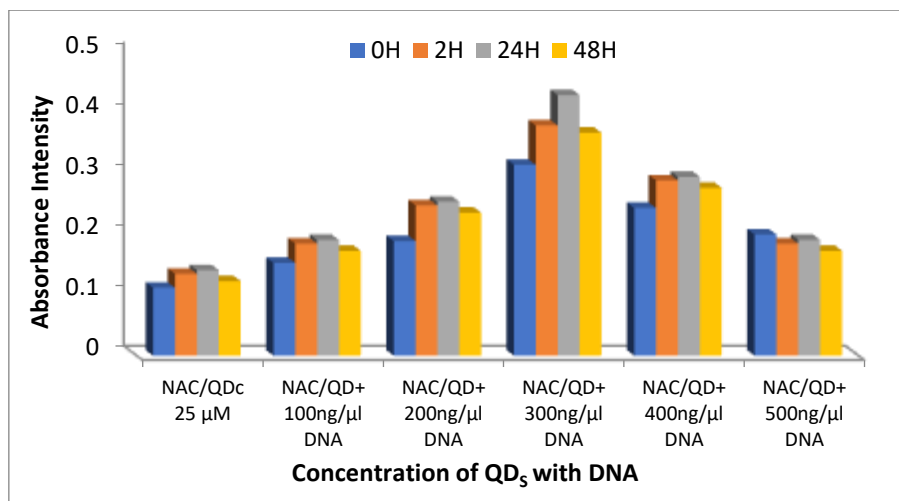
The QDs absorption spectrum for the damaged DNA was studied at different concentrations of DNA (100, 200, 300, 400 and 500 ng/ μ l). The prepared mixture was assessed at different times (incubation time) (0h, 2h, 24h and 48h) after conjugation between QDs + DNA. As shown in Figure 7(a, b, c) and Table 2, the DNA-conjugated NAC-CdTe QD sample absorbance intensity changed with different incubation time at room temperature. Because in the NAC-CdTe QD sample, the excess positive charge of cadmium was attractive to the different negative charges of phosphate sets in the DNA as a result of the difference in DNA concentrations, thus different number of attractive electricity forces led to the differences in intensity of absorbance and fluorescence. The best result of absorption spectrum intensity was at 300ng/ μ l concentration of an incubation time of 24h, suggesting that the NAC-CdTe QDs reacted with the DNA to actually form a complex. This result agrees with that of Wang et al. [24].



a



b



c

Figure 7: The relationship between absorbance intensity of (a) NAC-capped CdTe QDs at concentration of 1μM (b) NAC-capped CdTe QDs at concentration of 10μM (c) NAC-capped CdTe QDs at concentration of 25μM, with different concentrations of DNA and at different incubation times.

Table 2: The absorbance intensity of the different DNA concentrations at different incubation times

TIME	Sample NAC/CdTe 1μM	NAC/QD+DNA 100 ng/μl	NAC/QD+DNA 200 ng/μl	NAC/QD+DNA 300 ng/μl	NAC/QD+DNA 400 ng/μl	NAC/QD+DNA 500 ng/μl
0 h	0.066	0.080	0.189	0.155	0.111	0.126
2 h	0.079	0.114	0.248	0.185	0.140	0.152
24 h	0.084	0.124	0.245	0.195	0.150	0.162
48 h	0.067	0.102	0.236	0.173	0.128	0.140
TIME	Sample NAC/CdTe 10μM	NAC/QD+DNA 100 ng/μl	NAC/QD+DNA 200 ng/μl	NAC/QD+DNA 300 ng/μl	NAC/QD+DNA 400 ng/μl	NAC/QD+DNA 500 ng/μl
0 h	0.091	0.141	0.153	0.191	0.140	0.174
2 h	0.079	0.182	0.188	0.231	0.191	0.211
24 h	0.085	0.188	0.194	0.237	0.197	0.217
48 h	0.067	0.170	0.176	0.219	0.179	0.199
TIME	Sample NAC/CdTe 25μM	NAC/QD+DNA 100 ng/μl	NAC/QD+DNA 200 ng/μl	NAC/QD+DNA 300 ng/μl	NAC/QD+DNA 400 ng/μl	NAC/QD+DNA 500 ng/μl
0 h	0.112	0.153	0.189	0.315	0.244	0.200
2 h	0.135	0.185	0.248	0.380	0.289	0.185
24 h	0.141	0.191	0.245	0.430	0.295	0.191
48 h	0.123	0.173	0.236	0.368	0.277	0.173

3.5.2- Fluorescence of the NAC- CdTe QDs to detect reference and defective DNA:

Fluorescent spectroscopic properties of the NAC-capped CdTe QDs, NAC-capped CdTe QDs with undamaged DNA, and with damaged DNA were studied to find out the ability and

efficacy of the NAC-capped CdTe QDs to detect the damaged and the undamaged DNA. To do so, 25 μ M of the QDs were mixed with the best concentration conjunct of DNA (300ng/ μ l). In the same concentrations, the damaged DNA was mixed with NAC-capped CdTe QDs. The mixtures were assessed using the fully automatic ELISA reader to measure their fluorescence spectra, as shown in Figure 8. It can be noted from the figure that the maximum fluorescence peak of NAC-capped CdTe QDs spectrum was shifted from 550 to 540 nm by the interaction with the undamaged DNA; and the fluorescence maxima peak of the NAC-capped CdTe QDs was shifted from 550 to 535 nm by the interaction with the damaged DNA. Comparing these values declares a shift of 15 nm for the undamaged DNA sample and 10nm for the damaged DNA sample.

The same figure shows that the FWHM of the fluorescent emission spectra for the damaged DNA samples is wider and more intense compared to that of the undamaged DNA samples. Through this result, CdTe /NAC can be used as a fluorescent probe to detect undamaged and damage DNA[25].

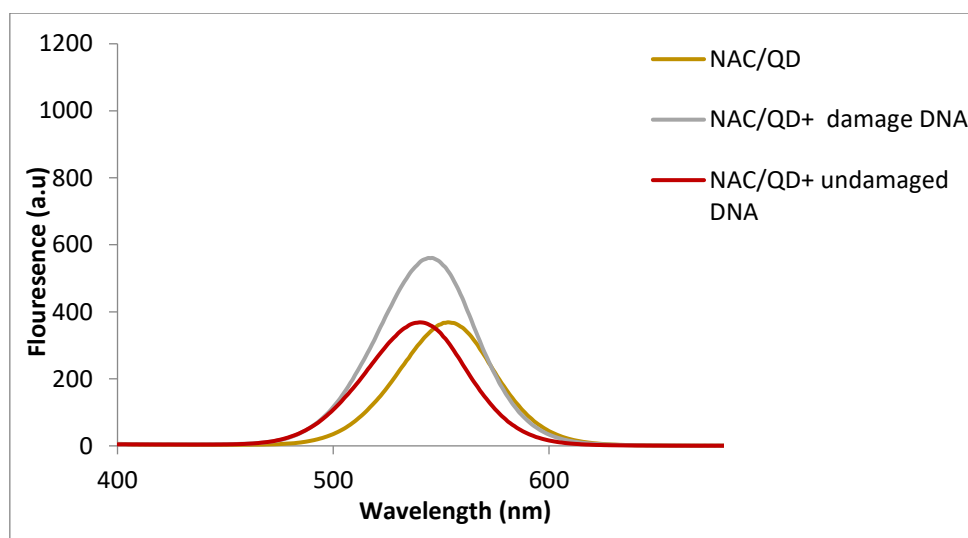


Figure 8: The fluorescence spectra of the NAC/QDs, the NAC/QDs with damaged DNA and NAC/QDs with undamaged DNA.

3.6- Conclusions

Aiming to improve the early detection of diseases marked by damaged DNA, including cancers, the present study concludes that the synthesized NAC-CdTe QDs could be employed in developing optical biosensors for various biomedical applications. The water-solubility and biocompatibility of the synthesized NAC-CdTe QDs are attributed to the polar carboxylic acid group of NAC on the surface of NAC-CdTe, and showed to have the potential to conjugate with various bio-macromolecular including nucleic acid (e.g. DNA). The synthesized nanocrystals were able to differentiate between damaged DNA (extracted from cancer patients' samples) and undamaged DNA (extracted from healthy individuals). Based on the obtained fluorescence results, the present study concluded that the prepared core/shell QDs could be employed as probes for diagnosing diseases that are marked by genetically disrupted DNA especially malignant tumors.

3.7-Acknowledgements

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