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Characterization of L-malic acid-capped CdS Quantum Dots and Examination of the Nanocrystal's Biosensor Ability

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Biochemistry, Molecular, and Cellular Biology Honors Thesis

Topic: Spectroscopic Characterization of Quantum Dots and Their Interactions with Amino Acids
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Abstract

Ligand-capped quantum dots are microscopic semiconductors commonly used in biomedical research and therapy for cell imaging and drug delivery. Despite the abundance of in vivo applications, the nature of interactions between the nanocrystals and the amino acids comprising human proteins remains insufficiently investigated. In this work, we report on the synthesis, ligand exchange, and characterization of the L-malic acid-capped CdS quantum dots via various spectroscopic techniques. We also assess the ligand-capped nanocrystals as potential probes for some amino acids. We determine that in the presence of L-cysteine, the L-malic acid-capped CdS quantum dots fluorescence signal quenches drastically, thus indicating a good potential the nanocrystals may have as the bio detectors of L-cysteine. Additionally, we review some of the previous experiments involving ligand-capped quantum dots and various analytes, including amino acids and their derivatives, pharmaceutical drugs, and bioactive compounds.
Introduction

Quantum Dots Preface

Quantum dots, also known as semiconductor nanocrystals, are artificial particles with unique optical and electronic properties due to quantum mechanics. Theorized in the 1970s and co-discovered in the early 1980s by two groups of scientists working independently, they soon became a prime topic in nanotechnology.¹ Today, quantum dots have found their application in various areas of physics, medicine, and ecology. From modern QLED TVs providing a brighter picture at a lower cost² to secure drug delivery systems for cancer patients³ and heavy metals detectors in water,⁴ quantum dots have already substantially contributed to many scientific accomplishments.

Such an abundance of applications stems from the semiconductor nature of quantum dots. Semiconductors are compounds that allow electron flow under certain conditions. Their conductivity is determined by the band gap, which refers to the energy range between the top of the valence band (the highest electron-occupied molecular orbital) and the bottom of the conduction band (the lowest electron-unoccupied molecular orbital).⁵ The band gap in semiconductors is much narrower than in insulators, although significantly wider than in conductors. When a valence electron absorbs enough energy to close the band gap, it becomes a charge carrier. While the general semiconductor properties remain applicable for quantum dots, there are some differences from their macroscopic counterparts.

Because their diameter rarely exceeds 10 nm, quantum dots cannot achieve consistent
valence and conduction bands - something the bulk semiconductors have. As a result, their band
gap varies with the size of a quantum dot. The energy required to transfer the electron to the
conduction band is inversely proportional to the size of the nanocrystal. Since energy and
wavelength are related by Planck's law, a change in the size of the quantum dot leads to a change
in the wavelength emitted. Smaller nanocrystals emit shorter wavelengths yielding purple and
blue colors, while larger ones emit longer wavelengths corresponding to red and orange colors.
Therefore, we can control the optical properties by adjusting the diameter of the quantum dot
while keeping other factors constant.

Another way to tune the characteristics of the nanocrystals is to alter their nature.
Researchers make quantum dots by using a sole starting material (typically, a semiconductor
from group 14 of the Periodic Table such as carbon, silicon, or germanium) or by combining two
elements (e.g., gallium arsenide, zinc selenide, or cadmium sulfide). The shape of the quantum
dot also has a significant effect on its electronic structure and optical properties. Any deficiency
in a crystal lattice of a semiconductor produces a "deep energy well" or a "deep trap" capable of
capturing the charge carrier either temporarily or permanently. Capping (attaching a ligand to
the surface of a nanocrystal) tends to enhance sterical stability by conserving the shape and size
of the individual dots and maintaining the band gap. Researchers often utilize such surface
modifications to detect a target substance by analyzing its interactions with the ligand of the
quantum dot-equipped probe.

**Literature Analysis: Various Analytes Detected by Ligand-Capped Nanocrystals**
Amino Acids and Derivatives

Carrillo-Carrión and collaborators investigated the detection of the carnitine enantiomers. This process is crucial for the pharmaceutical industry: L-carnitine is therapeutically effective, while D-carnitine has severe side effects. The researchers used L- and D-cysteine-capped CdSe quantum dots overcoated with ZnS to interact with the analyte molecules. As shown in Figure 1, Carrillo-Carrión and colleagues determined that the detection was selective. L-cysteine-capped CdSe (ZnS) quantum dots successfully spotted the presence of D-carnitine, while D-cysteine-capped CdSe (ZnS) quantum dots identified L-Carnitine. In both cases, a fluorescence quench signified the detection. The limits of detection were the following: 0.013 mM of L-carnitine with D-cysteine-capped CdSe (ZnS) and 0.016 mM of D-carnitine with L-cysteine-capped CdSe (ZnS). The main benefit of the cysteine-capped CdSe quantum dot probe is the high reproducibility of the system.

Figure 1 (Adaptation). Suggested mechanism of interaction between D-carnitine and L-cysteine-capped CdSe (ZnS).
Zare and Tashkhourian also chose cysteine enantiomers as ligands for their nanocrystals. The researchers constructed L- and D-cysteine capped CdSe quantum dots to investigate the detection of tryptophan enantiomers. Zare and Tashkhourian demonstrated that L-tryptophan quenches the L-cysteine capped CdSe nanocrystal fluorescence signal, and D-tryptophan acts accordingly on the D-cysteine-capped CdSe quantum dot signal (see Figure 2). The detection limits in the presence of 100 folds of another enantiomer were 4.4 and 4.8 nm for L- and D-tryptophan, respectively. The proposed method has high selectivity towards tryptophan enantiomers in the presence of various organic compounds and metal ions. This method may be preferable in medicine due to its accuracy and applicability for serum samples.

![Figure 2 (Adaptation). Suggested mechanism of interaction between the tryptophan enantiomers and cysteine ligands of CdSe quantum dots.](image)

Feizi and colleagues took advantage of cysteine as a capping agent as well. The scientists synthesized L- and D-cysteine-capped CdTe nanocrystals to detect tyrosine enantiomers known for their different functionality. They applied both fluorescence and circular dichroism.
techniques to identify each enantiomer. As portrayed in Figure 3, the L-cysteine-capped CdTe nanocrystals demonstrated a high selectivity towards the analyte compared to other amino acids comprising human proteins tested at the same concentration as tyrosine enantiomers. The main advantage of the proposed recognition method is the low limit of detection for L-tyrosine and D-tyrosine compared to the current techniques: 1.5 µM and 1.6 µM, respectively.

![Combined CD spectra of L-amino acids (50 µM) in the presence of L-cysteine-capped CdTe quantum dots.](image)

Gao and colleagues equipped the core-shell CdSe/CdS quantum dots with N-Acetyl-L-cysteine ligand. They aimed to come up with a probe capable of distinguishing the enantiomers of tyrosine, a nonessential amino acid. L-tyrosine is a precursor of several neurotransmitters often taken as a food supplement, while D-tyrosine is a biochemical reagent used in protein dynamics investigations. By obtaining the fluorescence spectra, the researchers determined that L-tyrosine causes a drastic quench, while D-tyrosine does not impact the
fluorescence signal (see Figure 4). Furthermore, the quantum dot sensor has demonstrated a high selectivity for L-Tyrosine compared to other amino acids and other potentially interfering compounds. The two main benefits of the proposed chiral recognition method are the low cost and low analysis time.

**Figure 4 (Adaptation).**\(^6\) Fluorescence emission spectra \((\lambda_{exc} = 380\ \text{nm})\) of N-Acetyl-L-cysteine-capped CdSe/CdS quantum dots in the presence of the increasing amounts of L-Tyrosine (A) and D-Tyrosine (B).

**Drugs**

Ngamdee and colleagues constructed a sensor from cysteine-capped cadmium sulfide quantum dots. They used two chiral thiol molecules to test the sensor: D-penicillamine (DPA) and L-penicillamine (LPA).\(^7\) The original circular dichroism spectrum of each molecule shows very little light rotation. However, as depicted in Figure 5, when the cysteine-capped cadmium sulfide quantum dot-derived sensor is applied, the spectra of DPA and LPA demonstrate strongly amplified signals that are mirror images of each other. The selective detection of enantiomers is important because LPA is clinically toxic, while DPA has many pharmaceutical applications. The
limits of detection for LPA and DPA were 0.74 µM and 0.49 µM, respectively. The main advantage of the sensor is the ability to test untreated urine samples and provide reliable results.

Figure 5 (Adaptation).\textsuperscript{17} The CD spectrum of 20 µM LPA and DPA with and without 0.04 mg mL\textsuperscript{-1} cys-CdS quantum dots.

The research group led by Anand applied the same type of quantum dots as those synthesized by Ngamdee and colleagues to a different target. The researchers studied the interactions between L-cysteine-capped CdS nanocrystals and the broad-spectrum antibiotic tetracycline.\textsuperscript{18} Tetracycline is widely used in pastoral farming to prevent livestock infections and enhance growth. Due to its adverse effects on humans, it is vital to have efficient detection methods. Anand and collaborators developed a quantum dot-based sensor that quenches its signal in the presence of tetracycline see figure 6). The limit of detection turned out to be as low as 7.78 µM. The constructed probe demonstrated an excellent selectivity compared to other antibiotics except for those belonging to the tetracycline family. In the case of chlortetracycline
and oxytetracycline, the fluorescence quench was smaller than for tetracycline. The main benefits of the proposed method are simplicity and low processing times.

Figure 6 (Adaptation). Combined fluorescent spectra of L-cysteine-capped CdS quantum dots in the presence of various concentrations of tetracycline (15 to 400 µM).

Masteri-Farahani and Mollatayefeh constructed D-cysteine-capped CdSe nanocrystals aiming to selectively detect L-morphine, which is an analgesic from the opioid family. As portrayed in Figure 7, the scientists have demonstrated that the fluorescence signal of D-cysteine-capped quantum dots quenches in the presence of L-morphine. The limit of detection for L-morphine was determined to be 0.06 µM. The constructed sensor probe is enantioselective, meaning it successfully detects L-morphine in the presence of its D-enantiomer. The main benefit of the proposed detection method is its low cost and short processing time.
Figure 7 (Adaptation). Proposed mechanism of the interaction between L-cysteine (to the left)- and D-cysteine (to the right)-capped CdSe quantum dots with L-morphine.

Unlike the previously mentioned research groups investigating the detection of one particular analyte, Delgado-Pérez and colleagues set a more ambitious goal. The researchers tried to detect several chiral drugs such as aryl propionic acids (APAs), ketoprofen (KP), naproxen (NP), flurbiprofen (FP), and ibuprofen. To accomplish the target, they developed an optical sensor by capping CdSe/ZnS quantum dots with N-Acetyl-L-cysteine methyl ester. Every drug resulted in a CD and fluorescence quench when assayed with the quantum dot-derived sensor. Figure 8 displays the quenching of the quantum dot fluorescence signal caused by S-ketoprofen. R- and S-enantiomers could be distinguished based on their spectra, which provides a selectivity that is so important in the pharmaceutical industry. The study did not purport to determine the limits of detection. The main advantage of the results is the derivation of the concentration equations based on the strength of a signal decay for each drug.
Figure 8 (Adaptation).\textsuperscript{20} Fluorescence spectra ($\lambda_{\text{exc}} = 420$ nm) of N-Acetyl-L-Cysteine Methyl Ester-capped CdSe/ZnS quantum dots ($9.36 \times 10^{-8}$ M) titrated with the S-enantiomer of ketoprofen.

Bioactive Compounds and Vitamins

Wang and colleagues used the dual-emitting sensor to detect folic acid (a B9 vitamin), whose deficiency is associated with fetal defects, cardiovascular diseases, and cancers.\textsuperscript{21} The researchers fabricated a detector from ZnS quantum dots codoped with Copper and Manganese ions. With the addition of folic acid, the fluorescence of the Mn\textsuperscript{2+} dopant enhanced, while the Cu\textsuperscript{2+} emission quenched. The analyte sensing mechanism is depicted in Figure 9. The probe was selective for folic acid, successfully sensing it in a solution containing multiple potentially interfering substances, including metal ions. The limit of detection turned out to be as low as 6 nM. The proposed nanocrystal-based sensor is superior to other methods due to its relative simplicity in synthesis.
Noipa and collaborators utilized Fe$^{3+}$-decorated Cys-CdS nanocrystals to construct a sensor capable of detecting the pyrophosphate (PPi) bioactive molecules. A product of ATP hydrolysis, pyrophosphate regulates various metabolic reactions, providing valuable information for cancer research. As Figure 10 reflects, pyrophosphate quenches the fluorescence signal when interacting with the nanocrystal probes. The limit of detection for the analyte turned out to be 0.11 µM. The nanocrystal probe demonstrated a high level of discrimination against other common anions, with pyrophosphate being the most effective quencher, indicating a high level of selectivity. The main advantage of the constructed sensor is its ability to detect the analyte at the micromol range in patients' urine samples.
Cumulative fluorescent spectrum of Fe³⁺-decorated Cys-CdS quantum dot dots in the presence of different pyrophosphate concentrations.

Experimental Background

Scientists frequently choose amino acids as ligands because of their great variety corresponding to a wide range of affinities to various materials. Polar and nonpolar, positively and negatively charged, amino acids can interact with numerous substances. Due to that factor, the same amino acid-capped nanocrystal (for example, L-cysteine-capped CdS quantum dot) may detect completely different objects such as the broad-spectrum antibiotic tetracycline and the toxic metal arsenic. Typically, an interaction between the quantum dot and the analyte modifies the fluorescence spectrum. This change in the fluorescence intensity of a sample may be positive (an enhancement) or negative (a quench). Other ways to determine the interaction include obtaining ultraviolet-visible (UV-Vis) or circular dichroism (CD) absorption spectra. In our experiment, we used all three techniques to collect the data.

The purpose of the study was to gain more knowledge regarding the nature of interactions between L-malic acid-capped cadmium sulfide (CdS) quantum dots and some of the amino acids...
comprising human proteins. Although there is not much information on the potential adverse effects on the human body, scientists commonly use CdS quantum dots in bioimaging and drug delivery.\textsuperscript{24-26} Previous toxicity studies demonstrated that CdS quantum dots slow the growth of offspring and cause abnormalities in the liver and kidneys in mice, along with affecting the cardiac development in zebrafish.\textsuperscript{27,28} While this study does not purport to address the toxicity issue, we try to identify and describe such interactions.

**Materials and Methods**

All chemicals used to synthesize quantum dots, ligand exchange reactions, and titrations were of reagent grade and were used without further purification. The deionized 18.2 MΩ water was provided by the Millipore Milli-Q water purification system. The Branson 2210 ultrasonic cleaning bath was used to accelerate the dissolution of amino acids in methanol. All circular dichroism (CD) readings were obtained on a Jasco J-1500 CD spectrometer. The ultraviolet-visible (UV-Vis) spectra were collected on a Jasco V-650 spectrometer. The fluorescence data were gathered by using a Jasco FP-8300 spectrometer. All three spectrometers were equipped with the Jasko Spectra Measurement program.

The synthesis of L-oleic-capped CdS quantum dots was previously described.\textsuperscript{29} Briefly, cadmium oxide (CdO), oleic acid, and organic solvent octadecene were mixed in a flask. The mixture was heated to 290°C under nitrogen flow while stirring. A separately made sulfur solution was quickly injected into the cadmium solution flask. The temperature was lowered to 260°C to allow the formation of the crystals. After five minutes, the sample was cooled down to room temperature and further purified.

The newly synthesized quantum dots dissolved in cyclohexane underwent a ligand exchange - a step crucially important for adjusting their properties.\textsuperscript{30} L-oleic acid was replaced
by L-malic acid. In short, the reaction took place on a stir plate in the deoxygenated nitrogen atmosphere. The sample remained in the darkness throughout the exchange process to prevent the degradation of the quantum dots. Tetramethylammonium hydroxide (TMAH) facilitated the replacement bringing the quantum dots from cyclohexane to methanol. Following the separation of the layers, the quantum dots dissolved in methanol were collected. The results of the CD spectroscopy were used to confirm the ligand exchange by comparing the experimental spectrogram of the L-Malic acid-capped quantum dots to the corresponding theoretical spectrogram. The quantum dot methanol solutions were kept with no access to sunlight at 4°C to prevent degradation.

Each amino acid solution used in titrations was prepared in a standard way. The necessary amount of amino acid to achieve the desired concentration was dissolved in deionized water. The dissolution of the sample was expedited by vortexing, followed by the sonication in the water bath. The amino acid solutions remained in a dark place at room temperature between the experiments. For all experiments involving UV-Vis or fluorescence spectroscopy, the baseline data were obtained before the data collection. Methanol was the baseline sample for the UV-Vis spectroscopy, while air served the analogous role for the fluorescence spectroscopy. The CdS quantum dot samples were titrated with the amino acid solution of interest. After each new aliquot was added, the cuvette was inverted to stir the mixture before obtaining any readings.

Following the completion of each experiment, the UV-Vis and fluorescence data were analyzed by the Jasco Spectra Analysis and Microsoft Excel programs. The Jasco Spectra Analysis program was mainly utilized to characterize the fluorescence data. The full-width half maximum (FWHM) value of the deep trap and maximum intensities for the band gap and deep trap were determined for each data point. Microsoft Excel was used to plot the absorbance and
fluorescence corresponding to each amino acid aliquot on the same graph.

**Results and Discussion**

**Characterization of L-malic acid-capped CdS quantum dots**

We obtained the L-malic acid-capped CdS quantum dots in a ligand exchange reaction and confirmed their chirality by CD spectroscopy. The CD spectrum of the directly obtained quantum dots was almost identical to the reference sample. Both CD spectra had three distinct negative peaks at 345, 395, and 440 nm and three clear positive peaks at 320, 360, and 420 nm. This observation is in line with the previous findings demonstrating that the optical activity of the nanocrystals capped with chiral ligands strongly depends on the composition of the quantum dots.\textsuperscript{31, 32}

Since the core of the quantum dot heavily influences the circular dichroism spectrum, we also examined the absorption of the L-malic acid-capped CdS quantum dots via UV-Vis spectroscopy. Based on the absorbance spectrum demonstrated in Figure 11, we applied the Beer-Lambert law to calculate the molar concentration of the quantum dots in methanol. The molarity turned out to be $3.77 \times 10^{-6}$ mol L$^{-1}$. 
**Figure 11. Absorbance spectrum of L-malic acid-capped CdS quantum dots dissolved in MeOH (3.77x10^{-6} \text{ mol L}^{-1}).**

**Interactions between L-malic acid-capped CdS quantum dots and various amino acids**

**L-cysteine**

For L-cysteine and L-tyrosine, we performed the titrations twice with two different amino acid concentrations (1.00x10^{-3} \text{ mol L}^{-1} and 5.00x10^{-2} \text{ mol L}^{-1}). The trends barely observed for the lower concentration were considerably amplified for the higher concentration. Therefore, we report only the latter results. As shown in Figure 12, the FWHM values sharply increased with the addition of each new aliquot. Both the band gap max intensity and the deep trap max intensity plummeted as the titration continued. We observed the elimination of both of the peaks after the addition of the second aliquot.
Figure 12. To the left: combined fluorescence spectra of L-malic acid-capped CdS quantum dots dissolved in MeOH (3.77x10^{-6} mol L^{-1}) titrated with L-cysteine dissolved in diH_{2}O (5.00x10^{-2} mol L^{-1}). To the right: cumulative data table characterizing each individual spectrum.

**L-tyrosine**

L-tyrosine was not as effective in quenching the fluorescence signal as L-cysteine. As reflected in Figure 13, both the band gap and the deep trap maximum intensity values decreased steadily following the increase in the L-tyrosine total amount in the sample. However, we noted a distinct feature unique to L-tyrosine. The FWHM values did not change throughout the experiment. This consistency indicates that while L-malic acid-capped CdS quantum dots may not be a very sensitive probe for the amino acid of interest, the measurements are reported with a high precision.24
Figure 13. To the left: combined fluorescence spectra of L-malic acid-capped CdS quantum dots dissolved in MeOH (3.77x10^{-6} \text{ mol L}^{-1}) titrated with L-tyrosine dissolved in diH_{2}O (5.00x10^{-2} \text{ mol L}^{-1}). To the right: cumulative data table characterizing each individual spectrum.

**Sensing mechanisms of L-malic acid-capped CdS quantum dots**

L-cysteine is the only amino acid with a thiol functional group we tested. Because thiols are far more acidic than alcohols, their conjugate bases are stronger.\textsuperscript{33} The thiol groups compete with the hydroxyl groups of L-malic acid, capping the quantum dots.\textsuperscript{34} Thiols bind onto the nanocrystal's surface, inevitably rearranging the original ligands or replacing them.\textsuperscript{35} Such interaction promotes the coating of the surface of CdS quantum dots with the new L-cysteine ligand. Previous findings have also demonstrated that interactions between the carboxyl group of cysteine ligand and carnitine lead to the quenching of the fluorescence signal.\textsuperscript{12} We may theorize that the similar interactions involving the carboxyl group led to a drastic change in the fluorescence signal.

The aromatic L-tyrosine amino acid was unique due to the presence of the phenol group. Titrated at the same concentration as L-cysteine, it had a much milder effect on the fluorescence signal. This mildness reflects the fact that the side chain pKa value is greater for tyrosine (10.07) than for cysteine (8.18).\textsuperscript{36} This difference in pKa values implies that tyrosine is significantly more alkaline than cysteine. Therefore, when competing with L-malic acid, tyrosine is likely to induce fewer structural rearrangements leading to a slighter change in the fluorescence signal. Additionally, Gao and colleagues proposed that the tyrosine's phenol group forms hydrogen
bonds that may somewhat enhance the fluorescent signal.\textsuperscript{16}

**Conclusion**

By reviewing the previous experiments utilizing ligand-capped quantum dots as bio detectors, we further confirmed the great variety of applications existing in the field. We also classified the detected analytes into three main categories: amino acids and their derivatives, drugs, and bioactive compounds. Additionally, we discovered that many researchers choose cysteine as a capping agent to take advantage of the bonding abilities of the thiol functional group.

In the practical part, we characterized the freshly made L-malic acid-capped CdS quantum dots via the CD and UV-Vis spectroscopies. Finally, we examined the interactions between the quantum dots and two amino acids making up human proteins (L-cysteine and L-tyrosine). Based on the results of our study, we determined that L-malic acid-capped CdS quantum dots have the best potential to be used as a biosensor for L-cystine. The strong and rapid quench in fluorescence signal occurring in the presence of L-cysteine implies that L-malic acid-capped CdS quantum dots may be used as accurate detectors for L-cysteine.

**References**


