Chiral Quantum Dots
for Biomedical Applications
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<td>QDs</td>
<td>Quantum Dots</td>
</tr>
<tr>
<td>PL</td>
<td>Photoluminescence</td>
</tr>
<tr>
<td>CD</td>
<td>Circular Dichroism</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic Light Scattering</td>
</tr>
<tr>
<td>Cys</td>
<td>Cysteine</td>
</tr>
<tr>
<td>ibu</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>PT</td>
<td>Phase Transfer</td>
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<tr>
<td>QY</td>
<td>Quantum Yield</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulphoxide</td>
</tr>
<tr>
<td>CdSe</td>
<td>Cadmium selenide</td>
</tr>
<tr>
<td>CdS</td>
<td>Cadmium sulphide</td>
</tr>
<tr>
<td>ZnS</td>
<td>Zinc sulphide</td>
</tr>
<tr>
<td>ZnSe</td>
<td>Zinc selenide</td>
</tr>
<tr>
<td>Mn/Mn$^{2+}$</td>
<td>Manganese</td>
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<tr>
<td>ZnS:Mn</td>
<td>Manganese doped zinc sulphide</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle’s Medium</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti-Inflammatory Drug</td>
</tr>
<tr>
<td>NPs</td>
<td>Nanoparticles</td>
</tr>
<tr>
<td>NC</td>
<td>Nanoclusters</td>
</tr>
<tr>
<td>ODE</td>
<td>1-octadecene</td>
</tr>
<tr>
<td>Ex. wav.</td>
<td>Excitation wavelength</td>
</tr>
<tr>
<td>DOPA</td>
<td>3,4-dihydroxyphenylalanine</td>
</tr>
<tr>
<td>Con A</td>
<td>Concanavalin A</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
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Abstract

The use of quantum dots (QDs) for biological and sensing applications is continuing to rapidly develop across a range of scientific fields. Because of their small size, and tunable optical properties, QDs are ideal for sensing and imaging applications. Their high photoluminescence quantum yields, high photostability, narrow photoluminescence spectra, and broad ranges of absorbance encourages their use in biological imaging. Their large, active surface area and environment-sensitive photoluminescence promotes their application in a wide range of biomedical sensing systems. Inducing a chiral response in QDs enhances their biomedical application, where chirality is ubiquitous in such systems.

The main goal of this project is to develop chiral quantum dots for a new enantioselective drug sensing system. The project involves the synthesis, modification and biomedical application of cadmium-based and zinc-based QDs. Experiments were conducted to find the optimum QD structure, composition and coating concentration, to produce QDs with the highest degree of both photoluminescence and chiral response, as these factors are crucial for an enantioselective system to be successful. The application of these optimised aqueous, chiral QDs resulted in effective enantiomeric discrimination of R- and S-enantiomers of the anti-inflammatory drug, ibuprofen, where one enantiomer was observed to quench QD luminescence to a greater extent than the other.

The secondary goal of increasing QD biocompatibility resulted in the development of QDs with improved stability in biological media, which was achieved through overnight incubation of zinc-based QDs with BSA. Enantioselective cytotoxicity was then demonstrated for BSA-coated L-/D-cysteine zinc-based QDs on A549 cells, in vitro: QDs that were coated with D-cysteine were more cytotoxic than those coated with L-cysteine.
1. Introduction

1.1 Quantum Dots

1.1.1 Optical Properties

Quantum dots (QDs) are semiconductor nanocrystals that are typically 2-10 nm in size.\(^1\) These nanoscale particles have become increasingly important in science due to their size-dependent optical properties.\(^1\) Since the 1980s,\(^4\) semiconductor nanocrystals have been of keen interest to scientists across a broad range of fields,\(^5\) where these size-dependent properties have a series of applications, from technology\(^6\) to medicine.\(^7,\)\(^8\) Alternating the size of the QD changes the size of the band gap that spans the distance between the valence band and the conduction band of the particle, as demonstrated in Fig. 1.

![Figure 1. Demonstration of band gap increase due to quantum confinement effect; Reproduced from SigmaAldrich.com.\(^9\)](image)

As the diameter of the QD is increased, the size of the mentioned band gap decreases. This means that in a larger QD, there is a smaller amount of energy required for an electron to get from the valence band to the conduction band and thus a longer wavelength of light will be emitted, and vice versa. When a QD is exposed to photoirradiation, excited electrons will jump to the conduction band, as demonstrated in Fig. 1. An excited electron will leave behind a positively charged hole in the valence band.\(^10\) This electron-hole pair is known as an exciton.\(^10\) The fluorescent light that is emitted by QDs, following excitation, results from radiative recombination of these excitons, which allows photons to be emitted. The ratio of emitted photons to absorbed photons is formally known as the quantum yield (QY).\(^11\) The QY can be reduced by surface defects...
on the QDs which prevent this radiative recombination of excitons from occurring and thus causes trapping and consequent loss of photoluminescence (PL). QDs typically have high QYs, with methods being developed over the years to increase this attractive property, which will be discussed in Section 1.1.2.

QDs demonstrate their unique electronic and optical properties due to a phenomenon known as quantum confinement. When semiconductor nanoparticles are smaller than their Bohr exciton radii (1-5 nm) their energy levels are quantised, meaning the energy levels change from being continuous to being discrete. This phenomenon relates band gap size to QD size, and allows the previously described tunable PL (Figures 1 and 2.)

As well as the ability to modify the colour of the light emitted by QDs, they will, conveniently, emit light once they have been excited by any wavelength that is larger than their exciton emission wavelength. This can be attributed to their broad absorption spectra and large extinction coefficients. The emission spectra characteristic to size-tunable QDs are narrow, and symmetrical, (Fig. 2) with a large Stokes shift which is vital for discrimination of QDs from other fluorescent signals such as different QDs or autofluorescence from tissue.

As well as their impressive optical properties, QDs also boast high photo- and chemical stability, and are strongly resistant to photobleaching. The small size scale of these
nanocrystals means they have a high surface to volume ratio\(^1\) and are highly medium sensitive, which is ideal for analytical purposes.

### 1.1.2 Structure Dependent Properties of QDs

QDs are typically synthesized using elements from groups II-VI, III-V, or IV-VI of the periodic table,\(^1\) and can exist in many different structures and compositions. This work will be focused on core-shell QDs and “doped” QDs, and include cadmium-based and zinc-based QD materials.

Core-type QDs consist of a core semiconductor material, such as CdS, which, for example, have been synthesized by Moloney et al. in 2007.\(^{13}\) Likewise, Gallagher et al. have used a similar synthesis to produce CdSe core-type QDs using microwave irradiation.\(^3\) In both cases, these nanocrystal cores were then coated in penicillamine ligands, which stabilises the colloidal QDs, and induces a chiral response.\(^3,13\)

Because surface states exist at the surface of pure semiconductors, where excitons can become trapped, optical and electronic properties of the nanocrystals can be affected\(^5\) which can result in a decrease in PL, as described in **Section 1.1.1**. Initially, the core-shell QDs, were developed as a way to increase PL QYs of QDs.\(^{17}\) Such an increase can be induced through introducing a second semiconductor nanocrystal as a shell around the original semiconductor nanocrystal core, where the shell has a higher band gap than the core.\(^{17}\) This phenomenon is known as surface passivation, which is a chemical process where the energy levels inside the gap of the semiconductor (core) are eliminated, when the atoms are bound to a material of a higher band gap.\(^5\) Although the use of organic ligands as a coating in the core-type QDs may act as a method for surface passivation of the QDs,\(^{13}\) the core-shell composition allows for enhanced PL and increased QY.\(^{18}\) Applying passivation with a second semiconductor nanocrystal allows for radiative recombination of electrons and ensures a significant decrease in non-radiative trapping,\(^{19}\) which if present can be the cause of a reduced QY. Core-shell QDs have become the typical preparation for luminescent nanocrystal semiconductors, with Mahler et al. showing that increasing the thickness of the shell will cause a significant increase of the QY, to ca. 70 %.\(^{20}\) Core-shell QDs may also have an organic, hydrophobic ligand coating on their surface,\(^5,17\) which offers additional stabilisation in organic solvents, as well as the possibility of a ligand transfer.

The “doped” QDs are typically prepared using zinc-based, ZnS or ZnSe, QDs as a core.\(^{21-23}\) Unlike cadmium-based QDs, which can emit red light, zinc-based QDs emit UV-Vis blue or green light. This isn’t ideal for QD bioapplications, which will be discussed, in **Section 1.3.2**. Using Mn\(^{2+}\) as a dopant for ZnS-core QDs allows for an alternative, phosphorescent decay pathway which is red-
shifted when compared to the non-doped ZnS QDs. In 1994, Bhargava et al. demonstrated that the electrons in the d-shell of Mn$^{2+}$ interact potently with the electrons in the s- and p-shells of the zinc core, which establishes an effective energy transfer path and leads to an increase in PL at room temperature.

A representation of this emission pathway can be seen in Fig. 3, where the excited electron returns to the valence band via a manganese triplet state which is closer in energy to the ZnS conduction band, than the ZnS valence band, and thus is a more energy efficient pathway of relaxation.

As depicted in Fig. 3, excited electrons will jump to the zinc conduction band as usual, but upon relaxation will take a lower energy-requiring pathway via the manganese orbital, which results in the characteristic orange emission of manganese. Because the colour of light emitted from zinc-core QDs is determined by the presence of the Mn$^{2+}$ dopant, changing the size of these specific QDs will not affect the colour of light emitted.

### 1.2 Applications of Quantum Dots

The size dependent PL of QDs makes these semiconductor nanoparticles highly attractive as assays for biomedical sensing and biological imaging. Their ability to emit light, ranging from ultraviolet to near infrared, has caused their recent bloom in the industry of fluorescent sensing. Their symmetrical spectra also increase the quality of imaging, as it makes it much easier to separate the PL of the QDs from that of the analyte(s), or background fluorescence.

Organic fluorophores and fluorescent dyes are traditionally used for most chemical and biological sensing applications. Organic fluorophores, although useful as fluorescent probes, have emission spectra that are generally asymmetric and resonate across a range of frequencies that is considerably narrow. Absorption and emission spectra also tend to overlap, which results in large cross sections over this narrow range. This unfortunate characteristic limits their application as
cellular labels, where a single organic fluorophore may not be used for detection of multiple analytes. The low photostability of organic fluorophores also means that labelling applications using these molecules are limited to short-time experiments. Longer lasting experiments, such as the monitoring of a slow biochemical process, pose quite a challenge, as well as introducing the risk of lost time, money and material if the assay out lasts the fluorescence time of the organic fluorophore. Conversely, the inorganic composition of QDs deems them very stable and robust light emitters, which makes them an attractive fluorophore for sensing and long-lasting biolabelling applications. This is especially important for imaging thick cells and tissue over long time periods, where many optical samples must be collected without inflicting damage to the analyte. Because QDs are also highly sensitive to their environment, their use in sensing is very effective. The optimisation and development of QDs over the past few decades has shown remarkable results in their use in cell tagging and labelling, single molecule sensing, pH sensing and ion sensing, to name just a few.

1.3 Quantum Dot Materials Used

1.3.1 Cadmium-based QDs

The use of heavy metals, such as cadmium, for the preparation of QDs has been widely practised for many years, generally in the form of CdSe core-type or CdS/CdSe core-shell QDs. Because cadmium-based QDs have PL and UV-Vis spectra in the visible region, they are highly advantageous for sensing applications. Adjusting QD size, for example, through the growth of multiple monolayer CdS shells around a CdSe core, results in a red-shift with each additional monolayer shell, as well as the added advantage of an increased QY. This brightly luminescent, red-shifted light is particularly beneficial for biological imaging, where there will be no overlap with cellular autofluorescence, which emits in the blue/green region. However, cadmium is unfortunately a highly toxic heavy metal element and therefore is not a suitable material for some biological applications, as it kills cells.

1.3.2 Zinc-based QDs

Unlike cadmium, zinc is an essential component of the body. As a non-heavy metal element, zinc doesn’t display heavy metal cytotoxicity and would therefore be much more suitable for biological administration. On the other hand, zinc also differs from cadmium in its region of absorption and of emission. Because the UV-Vis and PL of zinc-based QDs lie in the UV and blue regions of the spectrum it overlaps with tissue absorption and autofluorescence, meaning its use
in a biological system wouldn’t be very effective. However, the above mentioned development of manganese-doping has enabled the production of zinc-based QDs with red-shifted emission towards the 600 nm proportion of the spectrum.\textsuperscript{37} The evolution of core-shell QDs also presented the ability to cap a heavy metal cadmium-based core with a non-heavy metal zinc-based shell, in the specific form of CdSe/ZnS core-shell QDs.\textsuperscript{10,17}

1.4 Chirality

1.4.1 Chirality in Life

Chirality is a term used to describe two mirror image objects which are non-superimposable.\textsuperscript{38} Chirality is present in most aspects of everyday life. Not only does it affect our senses, such as smell,\textsuperscript{39} but it also determines how the cells in our bodies respond to different enantiomeric molecules, such as certain food or drugs. Because opposite enantiomers will have different effects on binding cellular receptors,\textsuperscript{40} it is important that the correct enantiomer is used for a specific response. Enantiomeric food components, such as proteins and sugars, are naturally available in the enantiomer that is accepted by the receptors in our bodies.\textsuperscript{38} On the other hand, because many drugs are synthesised, they can often be available as racemic mixtures,\textsuperscript{40,41} or as diastereomeric mixtures,\textsuperscript{40} where both enantiomers, or all stereoisomers, are present. As only one of two given enantiomers will have the desired therapeutic response in the body, the ability to discriminate between enantiomeric drugs is vital. The corresponding enantiomer may be ineffective, effective to a lower extent, or in some cases it can be toxic.\textsuperscript{38,42} Although methods already exist for enantiomeric separation and analysis, such as HPLC and GC, these processes are expensive, time consuming and laborious.\textsuperscript{43} There is thus a high demand for a method of sensing that is cost-efficient, highly sensitive and has a wide range of applications.\textsuperscript{43}

1.4.2 Chiral Quantum Dots

Chirality can be induced in QDs by introducing chiral molecules, such as amino acids, as surface ligands on the QDs. Introducing such ligands not only induces a chiral response, but also makes the QDs highly water-soluble. This process of introducing chiral ligands is thus known as a phase transfer (PT). It is achieved by adding chiral, water soluble ligands as a coating to the colloidal QDs, which are dissolved in an organic solvent. This subsequently allows the QDs to be transferred from the organic phase to an aqueous medium.\textsuperscript{44} In most cases, the addition of the chiral ligand will cause a ligand exchange between the original organic ligand and the added hydrophilic ligand. The chiral, aqueous ligand used will generally have a thiol group present, as thiols are known to
be able to bind strongly to QD surfaces.\textsuperscript{45} Other examples of inducing chirality/hydrophilicity involve conjugation of the new ligand with the original, organic ligand via hydrophobic interactions,\textsuperscript{46} but this is beyond the scope of this project and will not be further discussed. This ability to induce both chirality and aqueous solubility to QDs opens the potential application of these highly luminescent nanocrystals to enantiomeric drug sensing, where the demand for chiral discrimination has received increasing attention in recent decades.\textsuperscript{47} The use of chiral QDs for enantioselective sensing was previously demonstrated by Freeman et al. The group used β-cyclodextrin-capped CdSe/ZnS QDs for the chiroselective optical discrimination of D- and L-amino acids, tyrosine and phenylalanine.\textsuperscript{48} The group was successful in this discrimination, with the several-week stability of their QDs prompting the ongoing development of chiral, water-soluble QDs for biomedical sensing.\textsuperscript{48}

1.5 Aims of Project
The main aim of this project is to produce a system of chiral QDs that can chiroptically discriminate between R- and S-enantiomers of ibuprofen. Ibuprofen is an NSAID that is vital to those who suffer from arthritis,\textsuperscript{49} and to the general population for pain relief and treatment of inflammation.\textsuperscript{69} The objectives include the determination of optimum QD structure and composition for sensing, where QDs must display a chiral response as well as high PL. Ligand concentration for phase transfer is also an important factor. Han et al. showed that using a higher concentration of ligand for phase transfer increased the luminescence of the QDs.\textsuperscript{43} As well as this, the optimum ligand structure will be determined. Delgado-Pérez et al. demonstrated the successful chiral discrimination of two NSAIDs using CdSe/ZnS QDs capped with N-acetyl-L-cysteine methyl ester,\textsuperscript{50} but were unsuccessful in discrimination of ibuprofen enantiomers using this system. Another aim of this project is to investigate the influence of chirality on the biological activity of ZnS:Mn QDs. These non-heavy metal QDs are to be used for cellular applications, where CdSe/CdS QDs have already been recorded as highly cytotoxic.\textsuperscript{35} The stability of the ZnS:Mn QDs will be tested in the organic and aqueous phases, in the absence and presence of oleylamine. Aqueous phase QD stability tests are also planned to be performed in absence and presence of BSA. Both of these moieties are thought to influence QD stability in different media. It is expected that the new QD materials will find important biomedical applications as chiral biosensors or imaging agents in biological systems.
2. Experimental Section

2.1 Materials Used
All chemical reagents were purchased from Sigma-Aldrich, except R- and S-ibuprofen which were supplied by Santa Cruz. All chemicals were analytical grade and were not subject to further purification. CdSe seeds were produced by Postdoctoral researcher Dr. X before commencement of this project.

2.2 Methods of Characterisation
Methods of characterisation for QDs include UV-Vis, PL, CD, DLS and TEM. UV-Vis spectroscopy was performed using a Varian/Cary 50 spectrophotometer. A Cary Eclipse spectrofluorometer was used for PL spectroscopy measurements. CD spectra were measured using a Jasco J-810 CD spectrometer operating under an N$_2$ flow of 5-8 L/min.

DLS was used to collect hydrodynamic radii of QDs, using the Zetasizer Nano ZS system. TEM of QDs was performed using a FEI Titan electron microscope without aberration correction, operating at a beam voltage of 200 kV. (Credit given where appropriate.) The samples for TEM were prepared using QD solutions that were diluted to 1 mg/10 mL and allowed to dry at room temperature on copper grids.

The cell viability assay was carried out using CyteLL Cell Imaging System and High-Content Screening (HCS) analysis. Here, pre-installed GE Cell Viability BioApp 2-color protocol was used at 10x magnification to estimate cell viability. This test allows the (toxic) effect of QDs in cells to be monitored through measuring the quantity of cell nuclei present in a sample. A depletion in the number of nuclei after incubation with QDs will confirm a toxic response, which has resulted in cell death.

2.3 Synthesis and Phase Transfer of Quantum Dots
2.3.1 Synthesis of 5 shell CdSe/CdS QDs
CdSe/CdS QDs were synthesized by a Postdoctoral researcher in the X Group, Dr. X. I was involved in each step of this synthesis but was not active in the injection of reagents as I had not been trained to do so. Synthesis was followed according to the procedure of Mahler et al.$^{20}$ This specific synthesis was altered once all five monolayer shells had been formed. After formation of the fifth monolayer shell the reaction was heated from 230 °C to 250 °C and samples were then extracted
after 0 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours. Samples were then characterised and analysed to determine the optimum heating time for luminescence and chiral properties.

2.3.2 Phase Transfer of 5 shell CdSe/CdS QDs

The following solutions were prepared and used for cadmium-based and zinc-based PTs:

- Cysteine solution: L-/D-cysteine (49 mg; 0.404 mmol), 1 M hydrochloric acid (39 μL), methanol (1.5 mL)
- Thioglucone solution: 1-thio-β-D-glucose (22 mg; 0.101 mmol), 1 M hydrochloric acid (100 μL), methanol (1 mL)
- 1 M KOH solution: (324 mg; 5.77 mmol), water (45 mL)

PT\(^{(1)}\) procedure was adapted from method reported by Martynenko et al.\(^{44}\)
PT\(^{(2)}\) procedure was adapted from method reported by Moloney et al.\(^{51}\)

(a) L-/D-cysteine\(^{(1)}\)

5 shell CdSe/CdS QDs (750 μL) were diluted with chloroform (750 μL), and precipitated using methanol (1 mL). Centrifugation was used to separate precipitated QDs from solution, which were redissolved in chloroform (750 μL). Cysteine solution (75 μL) was added to appropriate QD/chloroform solution, shaken, and left for 2 minutes. Cysteine-QDs were then reshaken and KOH solution (750 μL) was added, adjusting pH to 12. Samples were slowly inverted, allowing QDs to gradually transfer to aqueous phase. This phase transfer could be observed by the naked eye as the QDs are an orange colour.

(b) 1-thio-β-D-glucose\(^{(2)}\)

5 shell CdSe/CdS QDs (750 μL) were diluted with chloroform (750 μL), and precipitated using methanol (1 mL). Centrifugation was used to separate precipitated QDs from solution, which were then redissolved in chloroform (750 μL). Thioglucone solution (60 μL) was added to appropriate QD/chloroform solution, shaken, and left for 2 minutes. Thioglucone-QDs were then centrifuged to precipitate QDs, which were then dissolved in DMSO (1 mL). The sample was then centrifuged to ensure no undissolved material was present, as this would cause aggregate formation during sensing. Orange QD/DMSO supernatant was separated and remaining QD solid was redissolved in DMSO (1 mL). (This centrifugation, separation and redissolving was repeated to make two more samples of sugar-QDs in DMSO.)

2.3.3 Synthesis of ZnS:Mn QDs

Synthesis was done according to methods reported by Yu et al.\(^{36}\)
Two reaction flasks are referred to as Pot 1 and Pot 2.

Zinc chloride (0.4 g; 2.93 mmol), manganese chloride tetrahydrate (0.018 g; 0.09 mmol), and dibenzylamine (54 mL; 281 mmol) were added to Pot 1, which was degassed under vacuum at 120 °C for 2 hours. Pot 1 was then removed from vacuum, put under argon, and cooled to 50 °C.

Sulfur powder (0.6 g; 18.75 mmol) was added to Pot 1, which was reheated to 260 °C and left for 15 minutes, before being cooled to 160 °C. Added to Pot 2 was dibenzylamide (10 mL; 52 mmol), and zinc chloride (0.8 g; 5.87 mmol). Pot 2 was degassed under vacuum at 120 °C for 1 hour. Pot 2 (5 mL) was added to Pot 1 via syringe. Pot 1 was reheated to 260 °C, and left at this temperature for 15 minutes. Pot 1 was cooled to 160 °C before addition of ethanol to cool solution and precipitate QDs. QDs were washed with ethanol several times to remove excess sulphur, which was indicated by the elimination of yellow supernatant. After cleaning, QDs were dissolved in chloroform (20 mL) and separated into two viles. Sample 1 was left as described, Sample 2 was prepared as above with addition of oleylamine (1 mL; 3 mmol) to stabilise QDs in organic solution.

2.3.4 Phase Transfer of ZnS:Mn QDs

(a) L-/D-cysteine

ZnS:Mn QDs (250 μL) were diluted with chloroform (250 μL) and precipitated using methanol (1 mL). Centrifugation was used to separate precipitated QDs from solution, which were redissolved in chloroform (1 mL). Cysteine solution (100 μL) was added to appropriate QD/chloroform solution, and shaken for 2 minutes. Cysteine-QDs were separated from organic supernatant by centrifugation, and redissolved in methanol (2 x 1 mL), to remove any impurities. Cysteine-QDs were separated by centrifugation after both washings. KOH solution/water (100 μL/2 mL) was then used to dissolve aqueous QDs, adjusting pH to 12.

(b) 1-thio-β-D-glucose

ZnS:Mn sample: Sample 1: without oleylamine

Sample 1 of ZnS:Mn QDs (250 μL) was precipitated using methanol (1 mL). Centrifugation was used to separate precipitated QDs from solution, which were redissolved in chloroform (1 mL). Thioglucose solution (100 μL) was added to QD/chloroform solution, and the sample was shaken for 2 minutes. Thioglucose-QDs were separated from organic solvent by centrifugation, and washed with methanol (2 x 1 mL), to remove any impurities. Thioglucose-QDs were separated by centrifugation after both washings. DMSO (1 mL) was then used to dissolve aqueous QDs. The samples were centrifuged to remove any undissolved QDs, with this process being completed several times to get a clear QD solution.
2.3.5 Phase transfer of ZnS:Mn QDs with and without oleylamine

(a) L-/D-cysteine\(^{(2)}\)

ZnS:Mn samples: Sample 1: without oleylamine, Sample 2: with oleylamine

Samples 1 and 2 of ZnS:Mn QDs were transferred according to Section 2.3.4(a). Water (5 mL) and KOH solution (260 μL) were then used to dissolve aqueous QDs. Samples were prepared using the resulting solution (750 μL) and KOH solution (750 μL), centrifuging to remove any aggregates.

2.4 Investigation of Quantum Dot Properties

2.4.1 Investigation of Shell Number for CdSe/CdS QDs

The concentrations of each sample (0 mins, 15 mins, 30 mins, 1 hour, 2 hours, 4 hours) were determined using UV-Vis spectroscopy, with toluene used as the baseline. Samples of equal QD concentration were then transferred to the aqueous phase using L-/D-cysteine, according to Section 2.3.2(a), with additional cysteine (10 mg/mL) added for overnight incubation. After PT, CD of each L-/D-cys-QD sample (1.5 mL) was measured in cuvettes, using water as the baseline.

2.4.2 Investigation of Cysteine Concentration for CdSe/CdS QDs

PT was completed as outlined in Section 2.3.2(a), with alternating cysteine solution volumes being used. Three sets of samples were prepared using cysteine solution (75 μL), for the standard cysteine concentration of 3 mg/mL. The cysteine concentration of two of the “standard” samples was increased to 6 mg/mL and 13 mg/mL, through cysteine solution additions of 75 μL and 250 μL, respectively. These samples were then incubated overnight, in darkness at 4 °C. CD of each L-/D-cys-QD sample (1.5 mL) was then measured in cuvettes, using water as the baseline.

2.5 Biomedical Testing I – Enantiomeric Drug Sensing

2.5.1 5 shell CdSe/CdS QDs with 1-thio-β-D-glucose Ligand for Ibuprofen Sensing

Samples were prepared in cuvettes, using 30 μL QDs per 1 mL DMSO. A bulk solution was firstly prepared which was then distributed among the twelve cuvettes. R-/S-ibuprofen solutions were prepared in DMSO with concentrations of 100 mg/mL, and 500 mg/mL. Increasing volumes of ibuprofen were added to QD/DMSO solutions from 20-100 μL, yielding overall ibuprofen concentrations of 3 - 7 mM, and 15 - 85 mM, respectively. All samples were mixed and left to equilibrate for 1 hour. UV-Vis and fluorescence spectra were then recorded (400 nm excitation.) A second 21 hour measurement was also recorded, with samples mixed before measurement.
2.5.2 ZnS:Mn QDs with 1-thio-β-D-glucose Ligand for Ibuprofen Sensing

Samples were prepared in cuvettes, using 46.7 μL QDs per 1 mL DMSO. A bulk solution was firstly prepared and distributed among the twelve cuvettes, as before. R-/S-ibuprofen solutions were prepared in DMSO with a concentration of 100 mg/mL. Increasing volumes of ibuprofen were added to QD/DMSO solutions from 20-100 μL, yielding overall ibuprofen concentrations of 3 - 7 mM. All samples were mixed and left to equilibrate for 1 hour. UV-Vis and phosphorescence spectra were then recorded (300 nm excitation). A second 3 hour measurement, and a third 22 hour measurement were also recorded, with each sample mixed before measurement.

2.6 Biomedical Testing II – Enantiomeric Cytotoxicity

2.6.1 Optimisation of ZnS:Mn QDs for Cellular Stability

ZnS:Mn samples: Sample 1: without oleylamine, Sample 2: with oleylamine
BSA solution: (excess) 0.3 mg/mL in cell media
Cell media: DMEM with 10 % FBS

PT was completed according to Section 2.3.5(a), with L-/D-cysteine ligands. Two of each sample (1.5 mL) were prepared, with the second sample incubated with BSA solution (50 μL), in darkness, overnight at 4 °C.

Samples were then prepared for DLS measurements. Previously prepared samples were firstly centrifuged to remove any aggregates. Centrifuged QDs (100 μL) were then added to a cuvette with water (1 mL). The same sample preparation was then repeated with cell media instead of water.

2.6.2 ZnS:Mn QDs Cytotoxicity Test

QDs: ZnS:Mn QDs with L-/D-cysteine ligands, incubated overnight with 0.3 mg/mL BSA
Media: DMEM with 10 % FBS and 5 μg/mL gentamicin
Cells: A549 lung cancer cells

A bulk solution (1 mL) of media with QDs, 9:1, respectively, was prepared, having a QD concentration of 32 μM. An aliquot (0.5 mL) of this solution was taken and added to a separate Eppendorf, with media (0.5 mL), allowing a two-fold dilution. This was repeated several times, taking an aliquot of each subsequent solution to prepare a more dilute solution in a new Eppendorf, with the final concentrations ranging from 4 - 32 μM. The A549 cells were seeded in a 96-well plate, with media (100 μL), where cell density was 5x10^3 cells per well. Following overnight (24 hour) incubation at 37 °C, QD/media solutions were added to appropriate wells,
with a volume of maximum addition of 100 μL per well. Media was used as a negative control, and valinomycin (100 μM) was used as a positive control. The plate was then incubated at 37 °C for 24 hours. After this time, Hoechst dye was used to stain the nuclei a blue colour. Cytotoxicity was then investigated through a cell viability assay, with ten fields imaged per well. An average cell viability was calculated comparing data from the experimental sample and the negative control.

3. Results and Discussion

3.1 Synthesis and Studies of Quantum Dots

3.1.1 Cadmium-based QDs

Preliminary work was conducted by Dr. X, to synthesize CdSe/CdS QDs with 1-5 CdS monolayer shells. This was done according to the method reported by Mahler et al., whereby cadmium and sulphur precursors were added to a solution of CdSe seeds, oleylamine, oleic acid, and ODE, via hot injection. Small changes to the experiment included: a larger scale (17x), heating at 230 °C for growth of monolayer shells, as well as a sample being taken between the growing of each monolayer shell. The aim of this experiment was to determine which composition would have both a chiral response and a high PL. It was previously observed by the group that CdSe/CdS QDs with one monolayer shell have a high chiral response but very low PL. We wanted to see if growing more monolayer shells would increase the QY with each additional monolayer shell; which would be observed via an increase in PL intensity. It was also expected that the red-shift would increase with the subsequent increase in QD size. After organic synthesis, each QD sample was then transferred to the aqueous phase using L- and D-cysteine, as outlined in Section 2.3.2, according to the method by Martynenko et al. Cys-QDs were then incubated overnight with excess cysteine (10 mg/mL). This was done in order to ensure that the entire QD surface was coated by the ligand, as each of the five samples would have different surface areas due to the increase in size. Cysteine was used as the chiral ligand for each of the following composition/structure investigation experiments due to its known successful use as a ligand for PT, significantly due to the presence of its thiol group, which is known to strongly bind to QD surfaces.

CD characterisation carried out on the five samples showed a gradual decrease in chiral response with an increase in number of shells. This is represented by plotting the G-factor, \( g = \frac{\Delta \varepsilon}{\varepsilon} \) (1), of each sample, which can be seen in Fig. 4A. G-factor values were used as they are not
concentration-dependent and would thus omit any concentration-dependent difference, error or inaccuracy.

Figure 4. A: The dependence of the G-Value in the excitonic region of L-/D-cys CdSe/CdS QDs on the number of CdS monolayer shells. B: The dependence of PL intensity of L-/D-cys CdSe/CdS QDs on the number of CdS monolayer shells.

The chiral response observed is thought to be induced by a hybridisation and coupling interaction between the HOMO of the cysteine ligand and the valence band states of the CdSe core of the QD.\(^{42,55}\) The observed trend of a reduction in chiroptical response with an increase in size corresponds with research conducted by Ben Moshe et al., where the group observed this same trend, having used size-exclusion chromatography to separate different sized particles of core-type CdSe and CdS QDs.\(^{56}\) The particle diameters ranged from 0.9 ± 0.03 nm to 2 ± 0.5 nm.\(^{42,56}\) The G-factor spectra for each of the measured samples is available in Appendix 1.1.

The dependence of the PL intensity on shell monolayers can be seen in Fig. 4B. Unlike the CD of the different samples, the PL increases with each additional CdS monolayer shell, where both the L- and D-cysteine 5 monolayer shell samples have the highest intensity PL.

The plots in Figures 4A and 4B determined that 5 monolayer shell CdSe/CdS QDs would be the optimum structure for chiral sensing, as this structure gives the highest PL intensity as well as a chiral response. This structure should thus allow detection of enantiomeric discrimination during sensing.

The next aim was to investigate the effects of shell crystallinity on the chiral and PL properties of the QDs. As outlined in Section 2.3.1, 5 shell CdSe/CdS QDs were again synthesised via hot injection of sulphur and cadmium precursors onto CdSe seeds, with oleylamine, oleic acid, and ODE.\(^{20}\) After all five monolayer shells had been grown, the reaction was heated for 10 mins at 230 °C. The temperature was then increased to 250 °C. An initial sample was taken as heating
began (0 mins), then further samples were taken after specific times (15 mins, 30 mins, 1 hour, 2 hours, 4 hours). At this high temperature annealing of the QDs occurs, where the ions can rearrange themselves into the most energy-stable configuration. Because annealing causes an increase in crystallinity, such an increase is expected to correlate with a higher temperature and heating time (where ions have increased energy and time to find the most efficient configuration). The six time dependent samples were characterised using UV-Vis, PL and TEM. CD spectra were measured after PT with excess L-/D-cysteine (13 mg/mL; to ensure full surface coverage), following induction of a chiral response. UV-Vis and PL spectra of time-dependent organic QDs are available in Appendix 1.2.

G-factor dependence on heating time of the six samples can be seen in Fig. 5A, where the intensity of the G-factor initially may increase as far as 15 mins, and will then decrease with increased heating time. As heating time continued, the G-factor values of the L- and D-cysteine QD samples had approximately the same degree of optical activity, with a deviation of ca. 10%. The exact reason for this trend is unknown, but it is thought that the longer annealing time of the QDs causes a change in the crystallinity of the surface in such a way that the binding of the ligands is affected. This will determine the concentration of (cysteine) ligand that will bind during PT which will thus affect the degree of chirality induced by the bound chiral ligands. Please see Appendix 1.3 for all G-factor spectra.

Figure 5. A: The dependence of G-Value in excitonic region of L-/D-cysteine 5 shell CdSe/CdS QDs on heating duration of 5th monolayer shell during synthesis; B: The dependence PL of L-/D-cysteine 5 shell CdSe/CdS QDs on heating duration of 5th monolayer shell during synthesis.

The PL trend of the heating time-dependent QDs can be seen in Fig. 5B. This trend differs greatly to that observed in Fig. 4B, where the spectra of L- and D-cysteine QDs overlap perfectly. In Fig. 5B the L- and D-cysteine QDs are not identical as samples were measured after a number
of days, where PL will have decreased in intensity to a slightly different extent in different samples. This doesn’t affect relative trends, where the higher PL intensity of the 4 hour samples is clearly distinguishable. Once again, from this investigation the sample that displays both a chiral response and has a high PL emission is optimal for a chiral sensing application; with this sample being the L-/D-cysteine-5 shell CdSe/CdS QDs after 4 hours of annealing.

Finally, the chiral/PL dependence of concentration of chiral PT ligand was determined. When determining the chiral response/PL of shell thickness (Fig. 4) and heating time (Fig. 5), an excess of cysteine was used to ensure that the entire surface of each QD was fully coated in chiral ligand. Because the concentration of coating ligand has been reported to affect QD properties, the influence of cysteine concentration on QD optical properties (namely CD) was investigated.

The standard concentration of 3 mg/mL of coating ligand (L-/D-cysteine) was used to prepare samples, with additional cysteine (3 mg/mL, and 10 mg/mL) being added to both (L- and D-) samples after PT, with overnight incubation (as described in Section 2.4.2). The results were again documented using G-values, where concentration is not taken into consideration, and only the effect on chirality. These data can be seen in Fig. 6.

![Figure 6](image_url)

**Figure 6.** The dependence of G-Value in the excitonic region of 5 shell CdSe/CdS QDs on concentration of cysteine.

From Fig. 6 it can be confirmed that a lower concentration of cysteine will induce a higher chiral response in 5 shell CdSe/CdS QDs. This can be denoted by the higher intensity G-values corresponding to the lower concentrations of cysteine used for PT.

Han et al. reported this investigation of concentration dependence using β-cyclodextrin as the coating ligand for CdSe/ZnS QDs, for amino acid sensing. The group added high and low concentrations of β-cyclodextrin to the QDs and found that higher concentrations of the ligand increased the PL of the QDs. Because the investigation of optimum shell thickness showed 5
monolayer shells to have a distinctively higher QY than the samples with thinner shells, it was not necessary to find a way to increase the PL of the QDs. QDs with 5 monolayer shells had the lowest chiral response of the five samples, therefore it is more beneficial to increase the chiral response of the QDs, if possible. The data in Fig. 6 show that using the lower concentration of 3 mg/mL of cysteine induces a higher chiral response in the QDs than the higher concentrations of 6 mg/mL and 13 mg/mL do. Therefore, for the application of enantiomeric sensing, the optimum ligand concentration to use would be 3 mg/mL, where the 5 shell CdSe/CdS QDs, which already have a high QY and display a chiral response, will now display a higher chiral response, which will hopefully enhance the ability of the QDs to discriminate between chiral molecules.

TEM images of the 5 shell CdSe/CdS QDs were taken by Dr. X, after the time-dependent synthesis. Images in Figures 7A-D were taken for two samples; before heating (0 mins) and after 4 hours of heating at 250 °C. QDs are in the organic phase, dried from toluene solution.

Figure 7. A*: TEM image of 5 shell CdSe/CdS QDs, after 0 mins of annealing; B*: Size distribution of 5 shell CdSe/CdS QDs, after 0 mins of annealing; C*: TEM image of 5 shell CdSe/CdS QDs, after 4 hours of annealing; D*: Size distribution of 5 shell CdSe/CdS QDs, after 4 hours of annealing. *TEM imaging was performed by Dr. X.
Table 1. Size distribution data of annealing time dependence during CdSe/CdS QD synthesis.

<table>
<thead>
<tr>
<th>Annealing time</th>
<th>N Total</th>
<th>Mean (nm)</th>
<th>Standard Deviation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mins</td>
<td>520</td>
<td>4.95824</td>
<td>0.76866</td>
</tr>
<tr>
<td>4 hours</td>
<td>322</td>
<td>4.57662</td>
<td>0.64874</td>
</tr>
</tbody>
</table>

Images were taken of the first and last sample in order to examine any changes in morphology or size that would appear as a result of longer annealing time during synthesis.

It was observed that the extra annealing time had caused the shape of the QDs to change slightly, from a spherical to a more triangular-like shape. This could be caused by the generation of a more energy-efficient configuration of the ions in the QDs, which leads to the formation of lower-energy faces on the particles. The formation of faces would cause the morphology to deviate from the observed spherically-based shape in Fig. 7A to the more triangular-like shape that can be seen in Fig. 7C. The diameters of the two forms of 5 shell CdSe/CdS QDs are 4.96 nm ± 0.77 nm, and 4.58 nm ± 0.65 nm, for 0 mins and 4 hours, respectively (Table 1.) The fact that the luminescence of the 4 hour sample is significantly brighter than that of the 0 mins sample, after PT, may be as a result of this change in surface. It is possible that this potential generation of more energy-efficient faces on the 4 hour sample will have less surface defects, which could increase the ease of ligand transfer. The change in crystallinity may also result in a weaker quenching by water, thus enabling the QDs to retain a greater intensity of PL following PT.

Having completed the above investigations of shell size, annealing time, and coating concentration, it was observed that 5 shell CdSe/CdS QDs should be the best suited structure for enantiomeric sensing, due to the presence of a chiral response, coupled with high PL emission. Optimum annealing time for high PL, as well as the retained chiral response was seen to be 4 hours. G-factor spectra of cysteine concentration suggested that a lower concentration of cysteine on the 4 hour annealing sample of 5 shell CdSe/CdS QDs would produce a higher chiral response. Because the ideal chiral sensing system would have both a high chiral and high PL response, in the case of this investigation, 5 monolayer shell CdSe/CdS QDs annealed for 4 hours at 250 °C with a 3 mg/mL ligand coating concentration are expected to be an effective system for such an application.

3.1.2 Zinc-based QDs

ZnS:Mn QDs were synthesized according to the method described by Yu et al.,\textsuperscript{36} with hot injection of ZnS into ZnS:Mn mixture, with heating from 120-270 °C. These QDs were characterised using UV-Vis, PL, DLS, and TEM. Like the cadmium-based QDs, cysteine was used for optimum structure/composition experiments. Because ZnS:Mn QDs already have a high QY and high PL, as
a result of the manganese-doping, there is no need to modify the structure of these QDs. In this case, an element of the composition that can be changed is the presence of the organic coating-ligand. A general practice with ZnS:Mn QDs is to add oleylamine into the organic solution once the QDs have been synthesized.\textsuperscript{36} The QDs do not dissolve fully in chloroform, where a cloudy mixture is observed. Adding the long chain oleylamine ligands stabilises the organic QDs, yielding a clear solution.

![Figure 8](image_url)

**Figure 8.** A: PL of ZnS:Mn QDs with and without oleylamine, in chloroform. Ex. wav. 300 nm; B: PL of L-/D-cys ZnS:Mn QDs, with and without oleylamine, in water. Ex. wav. 300 nm.

Experiments were conducted to determine if the added oleylamine ligand was actually improving the properties of the QDs, especially once QDs had been transferred into the aqueous phase, where heavy metal-free zinc-based QDs are of most convenience. Figures 8A and 8B show the PL spectra of both organic and aqueous ZnS:Mn QDs. It can be clearly observed, in both figures, that the QDs have a higher PL when oleylamine is not present in solution. Although PL is higher in the absence of oleylamine, there is still a significant decrease in QY after PT, which can be observed by the decreased PL intensities. Freeman et al. have attributed this to the generation of surface traps for the electrons in the conduction band of the shell, which occurs from the reaction of QD surface states with water.\textsuperscript{10}
TEM images were also taken of the synthesized ZnS:Mn QDs, by Mr. X. These images can be seen in Figures 9A and 9B, where the QDs are of a spherical shape. QDs are in the organic phase, dried from chloroform.

![Figure 9](image)

**Figure 9.** A* TEM image of ZnS:Mn QDs; B* Size distribution of ZnS:Mn QDs in chloroform.

*TEM imaging was performed by Mr. X.

These QDs are observed to have a diameter of 5.3 nm ± 0.74 nm, which makes them larger in size than the 5 shell CdSe/CdS QDs presented in Figures 7A-D. This size difference is a result of the ZnS:Mn QDs not having size-dependent coloured light, as the emission comes from the manganese triplet state within the doped-core.24 Although the 5 shell CdSe/CdS QDs are smaller in size, they still emit a more red-shifted colour of light due to the different mechanism of emission, whereby electrons relax from the CdS valence band to the corresponding hole in the CdSe conduction band, as described in Section 1.1.1

### 3.2 Biomedical Testing I – Enantiomeric Drug Sensing

#### 3.2.1 5 shell CdSe/CdS QDs with 1-thio-β-D-glucose Ligand for Ibuprofen Sensing

Once investigations of optimum QD composition and structure were complete, the 5 shell CdSe/CdS QDs were used for enantiomeric drug sensing. The main aim of this part of the project was to design a sensing system for discriminating between R-/S-ibuprofen enantiomers. Instead of using L-/D-cysteine for this system, 1-thio-β-D-glucose was used as the chiral ligand for PT. Previously, Delgado-Pérez et al. designed a sensing system for NSAIDs using CdSe/ZnS QDs capped with N-acetyl-L-cysteine methyl ester.50 Although the group succeeded in discriminating between enantiomeric ketoprofen and fluriprofen, they did not observe any difference between R- and S-ibuprofen.50
A. L-/D-cysteine  
![L-cysteine](image1.png)  
B. R-/S-ibuprofen  
![R-ibuprofen](image2.png)  
C. 1-thio-β-D-glucose  
![1-thio-β-D-glucose](image3.png)

**Figure 10. Structures of used PT ligands (A, C) and target enantiomers for quenching (B)**  
(Credit: ChemBioDraw Ultra 14.0)

As well as the findings of Delgado-Pérez et al., L-/D-cysteine were preliminarily used by fellow group member, Ms. X, as PT ligands for this cadmium-based sensing system, and although ibuprofen quenching was observed, there was no enantiomeric discrimination (data not shown.) Therefore, it was apparent that these cysteine-structured ligands did not cause chiral discrimination with ibuprofen before commencing the described system design. Because enantiomeric separation of drugs, and other chiral molecules such as amino acids, is commonly performed using a sugar molecule, cyclodextrin, it was decided that a sugar molecule may induce better chiral discrimination than an amino acid, in such a system. Thus, 1-thio-β-D-glucose was used as the chiral ligand for PT of 5 shell CdSe/CdS QDs, with the thiol group an essential component for QD-binding.

Two different ibuprofen concentrations were used on the sensing system; 100 mg/mL and 500 mg/mL. Because ibuprofen is not soluble in water, but is soluble in DMSO, PT of QDs was achieved with the chiral sugar ligand according to Section 2.3.2(b), where instead of dissolving QDs in water, like is done with L-/D-cysteine, the sugar-coated QDs were precipitated from solution and then dissolved in DMSO. This was done in order to avoid any phase separation that may occur if QDs are dissolved in a different medium to that of the overall sample. Sugar-coated QDs were then centrifuged to remove any QDs that were not in solution and may aggregate and cause error in results. After solution in DMSO, sensing was completed as outlined in Section 2.5.1, with the samples observed under UV-light directly after preparation and again after 1 hour. Under UV-light (365 nm) there was a visible quenching of PL in the R-ibuprofen samples of concentrations 50-85 mM, where QDs could be seen to have precipitated from solution and had gathered at the bottom of the cuvette. This suggests that the higher concentration of R-ibuprofen
molecules may cause a significant shielding around the polar sugar groups, causing the QD to be precipitated out of the polar solvent. PL was measured before and after mixing solutions (Appendix 1.5, II), with production of the same trend showing that these precipitated QDs were also quenched. All S-ibuprofen samples were stable in solution, with no precipitation observed under UV-light. Neither was PL quenching detected 1 hour after addition of drugs. Figures 11A and 11B show 5 shell CdSe/CdS QDs with 1-thio-β-D-glucose ligand, in DMSO. (PL had decreased slightly when samples were re-measured after 21 hours, please see Appendix 1.5, I.)

**Figure 11.** A: PL of 5 shell CdSe/CdS QDs, 1 hour after addition of drugs. Ex. wav. 400 nm; B: Quenching curve of 5 shell CdSe/CdS QDs, 1 hour after addition of drugs.

It is clear that the R-ibuprofen samples quench the QDs, whilst the S-samples do not (1 hour after measurement.) After 21 hours the trend continued with R-samples being further quenched, and S-samples displaying a small degree of quenching (Appendix 1.5).

### 3.2.2 ZnS:Mn QDs with 1-thio-β-D-glucose Ligand for Ibuprofen Sensing

Like the CdSe/CdS QDs, once the optimum composition/structure of the ZnS:Mn QDs had been investigated, these QDs were applied to the ibuprofen sensing system, again using 1-thio-β-D-glucose as the chiral ligand for PT, and an ibuprofen concentration of 100 mg/mL. ZnS:Mn QDs used did not have oleylamine added after synthesis for reasons described in Section 3.2.1. Results are available in Figures 12A-14B. Interestingly, the opposite quenching effect was observed with the zinc-based QDs, than with the cadmium-based QDs, where the ZnS:Mn QD quenching was caused by interaction with S-ibuprofen. The interaction of the QDs with R-ibuprofen was seen to stabilise spontaneous quenching of the QDs over a 24 hour period. Figures 12A-14B show ZnS:Mn QDs with 1-thio-β-D-glucose ligand, in DMSO.
Figure 12. A: PL of ZnS:Mn QDs, 1 hour after addition of drugs. Ex. wav. 300 nm; B: Quenching curve of ZnS:Mn QDs, 1 hour after addition of drugs.

Figure 13. A: PL of ZnS:Mn QDs, 3 hours after addition of drugs. Ex. wav. 300 nm; B: Quenching curve of ZnS:Mn QDs, 3 hours after addition of drugs.

Figure 14. A: PL of ZnS:Mn QDs, 22 hours after addition of drugs. Ex. wav. 300 nm; B: Quenching curve of ZnS:Mn QDs, 22 hours after addition of drugs.

3.2.3 Potential Theory for QD Quenching with Ibuprofen

Figures 11A-14B display the cadmium-based and zinc-based QD quenching with ibuprofen enantiomers. Because the use of QDs for enantiomeric sensing, and the use of carbohydrates...
as the PT ligand for these systems, are both new fields, the published literature for the proposed quenching mechanisms is very limited. Carillo-Carrión et al. offered a mechanism for the interaction of D-carnitine with CdSe/ZnS QDs capped with L-cysteine, and vice versa.\textsuperscript{52} The group suggested that the carnitine molecule can interact with the appropriate cysteine ligand through carboxylic, amino and hydroxyl groups, because of the significant conformation of both enantiomers.\textsuperscript{52} This agrees with work of other research groups, where homochiral interactions are weaker than heterochiral ones,\textsuperscript{61} as well as mimicking the well-known three-point model for chiral binding.\textsuperscript{52} As previously described, Delgado-Pérez et al. have used CdSe/ZnS QDs capped with N-acetyl-L-cysteine methyl ester ligands to sense chiral NSAIDs, including ketoprofen. The quenching mechanism proposed by the group involves the NSAID binding to the N-acetyl-L-cysteine methyl ester ligand via the carbonyl amide group of the ligand, which breaks the bidentate binding of the ligand to the QD and causes surface reorganisation. Finally, the NSAID is proposed to bind directly to the surface of the QD via its carboxylate group, where the aromatic entity of the NSAID reduces QD fluorescence, thus increasing quenching.\textsuperscript{50} Choi et al. demonstrated quenching of PbS QDs capped with a thrombin-binding-aptamer with thrombin.\textsuperscript{62} The quenching was believed to be caused by a charge transfer from the functional groups on the thrombin protein to the QD surface.\textsuperscript{62} Whereas Kang et al. reported that gold nanoparticles capped with L-/D-penicillamine would allow heterochiral electron transfer between Au-surface and L-/D-DOPA molecules, which would in turn cause quenching of the NPs.\textsuperscript{63} CdSe/ZnS QDs capped with β-cyclodextrin were used by Han et al. to sense amino acid enantiomers, L-/D-tyrosine, where the fluorescence of the QDs was enhanced rather than quenched.\textsuperscript{63} The reason for this was thought to be via the generation of a new radiative pathway on binding of the L-/D-tyrosine with the β-cyclodextrin ligands, which were coating the entire surface of the QDs.\textsuperscript{63} Another possibility was that a non-radiative path was being suppressed.\textsuperscript{63} Finally, Earhart et al. assessed the biochemical activity of CdSe/ZnS QDs functionalised with dextran through addition of a lectin called Concanavalin A.\textsuperscript{64} Because Con A has binding specificity towards glucose and mannose, it would bind the dextran functionalised QDs and cause them to aggregate and consequently precipitate from solution.\textsuperscript{64} This time-dependent precipitation of QDs would cause a decrease in fluorescence of the sample, with quenching time taking ca. 4 hours.\textsuperscript{64} Taking the above research findings into consideration allows the suggestion of a quenching mechanism for the observed quenching of both 5 shell CdSe/CdS and ZnS:Mn QDs, each coated with 1-thio-β-D-glucose. The fact that opposite ibuprofen enantiomers were quenched using the
different QDs is a significant factor to be considered. Because the same PT ligand was used for both QDs (1-thio-β-D-glucose), this indicates that the quenching mechanism may not be determined by the coating ligand, or more importantly, by the specific enantiomer used for coating. If this was the case only one of the ibuprofen enantiomers would interact with the chiral ligand and thus the same ibuprofen enantiomer would be seen to quench both types of QDs. This therefore suggests that the interaction causing the PL quenching may occur via direct binding to the QD surface, such as suggested by Choi et al. Because the surface of the CdSe/CdS QDs differ from that of the ZnS:Mn QD surface, both in terms of structure and of composition, it is thus possible that each surface may have preferential binding for a different ibuprofen enantiomer. Delgado-Pérez et al. have already given a plausible mechanism for QD quenching with NSAIDs whereby the drug enantiomer binds the QD surface firstly via the chiral PT ligand. Therefore, a further possibility is that the 1-thio-β-D-glucose ligand is the moiety that binds differently to both QD surfaces, which is instead what causes the different enantiomeric selectivity. Unlike the typically used amino acid ligands, 1-thio-β-D-glucose doesn’t have any carboxylic or amine groups for binding or interacting with enantiomers. Instead, the D-sugar has several chiral alcohol groups, as well as the thio-moiety that binds the QD surface (indicated in Fig. 15 by a “squiggly” line.)

The chiral sugar exists in a non-flat (chair) conformation, as can be seen in Fig. 15. The different QD surfaces will surely cause different 1-thio-β-D-glucose positioning/distribution, which in turn may have a different overall surface conformation of the chiral ligands. These different surface conformations could thus be the cause of the differential enantiomeric binding observed between the different QD surfaces.

The presence/absence of organic ligands during synthesis (oleic acid, oleylamine vs. none; CdSe/CdS vs ZnS:Mn, respectively), could also be a contributing factor to this proposed surface discrimination. Perhaps the presence of passivating ligand leaves more surface defects during PT procedure, and vice versa.
The TEM images (Figures 7A,D and 9A) show that these cadmium-based and zinc-based QDs, respectively, have different shapes, where the 5 shell CdSe/CdS QDs are more triangular-like after four hours of annealing, whereas the ZnS:Mn QDs are of a more spherical-shape, typically characteristic of so-called “spherical” QDs. These different shapes alone will cause a different distribution of (chiral) coating ligand which could easily lead to different enantiomeric selectivity (of the ibuprofen.) This could either be through something like the three-point model, or even just the overall shape of the coating ligands acting as a pseudo-binding site for a specific enantiomer.

3.3 Biomedical Testing II – Enantiomeric Cytotoxicity

3.3.1 Optimisation of ZnS:Mn QDs for Cellular Stability

After the investigation of the ZnS:Mn QDs as an ibuprofen sensing system, further investigation was done on these non-heavy metal QDs for cellular imaging applications. ZnS:Mn QDs with and without added oleylamine were transferred to the aqueous phase using L-/D-cysteine and were then investigated in both water and cell culture media, with and without BSA. Aggregation of QDs can greatly affect results when cytotoxicity and cell count tests are being undertaken. As mentioned in Section 3.2.1, ZnS:Mn QDs have a higher emission in both the organic and the aqueous phase when oleylamine is not added after synthesis. DLS measurements carried out on the above mentioned samples confirmed that the presence of oleylamine does not stabilise the QDs in water. It can be seen in Figures 16A and 16B that a higher degree of aggregation is present in the sample containing oleylamine.

![Figure 16](image)

**Figure 16.** A: DLS of L-cys ZnS:Mn QDs in water, with oleylamine; B: DLS of L-cys ZnS:Mn QDs in water, without oleylamine.

The ligands that coat hydrophilic QDs stabilise the NPs in solution. This is because these equally charged, identical ligands induce electrostatic repulsions that keep neighbouring QDs from
interacting. When hydrophilic QDs come into contact with high salt concentrations, the salt ions can shield this electric field by interacting with charged groups on the stabilising ligands. Because cell media contains high concentrations of salt, this phenomenon allows QDs to come into close contact with one another and agglomerate, through induced dipole interactions. Some proteins, such as serum albumin, have been known to stabilise QDs through prevention of this aggregation. Because BSA is the most abundant protein in FBS, which is present in the used cell media (DMEM, 10 % FBS), addition of this protein to QDs will not have any negative effects on the cellular entities.

Figure 17. A: DLS of D-cys ZnS:Mn QDs without both oleylamine or BSA, in cell media; B: DLS of D-cys ZnS:Mn QDs without oleylamine, incubated overnight in BSA, in cell media.

As can be seen, in Figures 17A and 17B, overnight incubation of cys-QDs in BSA greatly reduces aggregation when QDs are in cell media. This means that incubating QDs in BSA before cellular application will greatly enhance their biocompatibility and accuracy.

All DLS spectra of both L- and D-cys QDs, with and without both oleylamine and BSA, in water and in cell media, can be seen in Appendix 1.6. Both L- and D-cys QDs aggregated in the presence of oleylamine and absence of BSA, whereas reduced/no aggregation was detected in absence of oleylamine and with prior incubation with BSA. Hydrodynamic radii of L-/D-cys QDs in cell media, after BSA incubation, were 7 nm ± 1 nm, for both enantiomers, with QDs incubated with BSA remaining monodispersed within the cell media. Conversely, L-cys and D-cys QDs that weren’t incubated in BSA aggregated together in cell media, and had hydrodynamic radii of 58 nm ± 11 nm, and 70 nm ± 9 nm, respectively. Following these results, QDs were incubated in BSA before cellular application. Enantiomeric differences were also observed when the chiral ZnS:Mn QDs were tested on cells.
3.3.2 Cytotoxicity Studies of ZnS:Mn QDs

The L-/D-cys QDs were mixed with DMEM containing 10% FBS, added to the A549 cell culture and incubated for 24 hours, with QD concentration ranging from 4 to 32 µM. After 24 hours the results in Fig. 18 were observed, where L-cys QDs displayed a much lower extent of cytotoxicity than the D-cys QDs, across the range of concentrations.

As expected, the zinc-based QDs showed a lower cytotoxic effect than cadmium-based QDs from literature.\(^{35}\) It was interesting that QDs coated with L-cysteine, the amino acid that is naturally utilised and accepted by our bodies, had the less cytotoxic response. Yu et al. used 1-thio-β-lactose ligands to coat CdSeS/ZnS QDs, where these QDs were applied as fluorescent labels for cells.\(^ {67}\) The group measured hydrodynamic radii (up to 8.2 nm) and performed a cell viability assay using these “polyvalent lactose-QDs”\(^ {67}\). The cytotoxicity measurement was performed on Hela cells in DMEM with 10 % FBS and 1 % penicillin/streptomycin. Twenty four hour incubation of Lac-QDs, with concentrations up to 0.5 mg/mL, with Hela cells, revealed next to no cytotoxicity.\(^ {67}\) Like the reduced cytotoxicity of L-cysteine observed in Fig. 18, the D-sugar was used by Yu et al. in their experiment, which is the sugar enantiomer naturally utilised/accepted by the body.

In two separate findings L-/D-GSH ligands were used as hydrophilic stabilising ligands for cadmium-based QDs\(^ {68}\) and gold-based NCs\(^ {69}\), by Li et al., and Zhang et al., respectively. Whilst Li et al. found CdTe-L-GSH QDs to have a higher degree of cytotoxicity after 24 hour incubation,\(^ {68}\) Zhang et al. observed the opposite effects with their NCs; where Au-D-GSH had a higher cytotoxic response following the same incubation time.\(^ {69}\)

These findings demonstrate a few possibilities of this opposite enantiomeric recognition. Most likely, the different cytotoxicity can be explained by the fact that different cell lines have been used in each experiment, and the type of cell is selective for a specific enantiomer. It could also have something to do with the material used and the natural versus unnatural enantiomer. A
stronger selectivity for the “natural” L-amino acid will have higher cytotoxicity when the material is cadmium, compared to when it is zinc; in which case the “unnatural” D-enantiomer may have a higher toxic response. These theories will need further experimental studies using different QD structures, compositions, and materials, to see if such trends hold. The findings described in this investigation, specifically using L-/D-cys-ZnS:Mn QDs, portray a possible trend where the use of the so-called “natural” enantiomer as the chiral ligand for PT of zinc-based doped-QDs may aid in making QDs more biocompatible and less cytotoxic.

4. Conclusions and Future Work

In this project I (alongside my fellow researchers) successfully developed the synthesis, and investigated the modification, induced chirality and water solubility of cadmium-based and zinc-based QDs. These chiral, aqueous QDs were then tested for potential biomedical applications; as sensing systems for enantiomeric NSAID, ibuprofen, as well as the zinc-based QDs being investigated for a potential in vitro cellular imaging application.

Using cadmium-based and zinc-based QDs as sensing systems for the same chiral drug resulted in the discovery of opposite enantiomeric selectivity for cadmium- and zinc-QD quenching. The proposed quenching mechanism involved the QD-surface-dependent formation of a bulk 1-thio-β-D-glucose conformation analogous to a binding-site, where a specific ibuprofen enantiomer will have a greater binding affinity towards this ligand conformation. Through this enhanced chiral binding, which can be summarised with the renowned “three-point model” for enantiomeric binding, one of the ibuprofen enantiomers will be able to bind the QD surface in a substantially greater concentration than the other enantiomer, which will not be able to readily access the surface through the enantioselective 1-thio-β-D-glucose binding-moiety. This proposed pseudo-binding-site formed by the selective binding of highly chiral 1-thio-β-D-glucose coating ligands is a result of the different QD surfaces associated with 5 shell CdSe/CdS QDs (after four hours of annealing) and ZnS:Mn QDs, without post-synthesis addition of oleylamine. The different structures and compositions of these QDs offer different binding site availabilities for the 1-thio-β-D-glucose surface coating, which should cause this proposed difference in ibuprofen enantiomer binding. The enantiomerically selective binding of R- and S-ibuprofen to 5 shell CdSe/CdS and ZnS:Mn QDs, respectively, may then go on to either block radiative recombination or favour a non-radiative pathway within the appropriate QD moiety.
Of course, these findings are still in very early stages and plenty more research is needed before any mechanism can be fully accepted and proven. As the project stands there is definitely an interaction of some sort occurring that allows enantiomeric discrimination. Future work will involve repeating these sensing experiments several more times, altering ibuprofen concentrations and using samples of enantiomeric mixtures, which will help to determine the source of the quenching.

The enhanced biocompatibility of ZnS:Mn QDs was achieved through incubation of QDs with BSA, and omitting the addition of oleylamine after synthesis. Results showed that the use of “natural” enantiomer could be a potential step forward to reducing cytotoxicity of QDs and promoting their use in cellular imaging. Future work for this application involves further analysis into the optimum PT ligand for reduced cytotoxicity, as well as any possible structure/composition/material comparisons. Also, detailed studies of these QDs in vitro using confocal microscopy and fluorescent lifetime imaging (FLIM) will be necessary to evaluate intracellular uptake and localisation, as well as optical parameters of QDs in various cell cultures.
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Appendix

1.1 The Dependence of the G-Value of CdSe/CdS QDs on the Number of Monolayer CdS Shells. Measured in the excitonic region. Cysteine concentration is 13 mg/mL.

A. 1 monolayer shell

B. 2 monolayer shells

C. 3 monolayer shells

D. 4 monolayer shells

E. 5 monolayer shells
1.2 UV-Vis and PL of Heating-Time Dependent 5 Shell CdSe/CdS QDs in Toluene

A: UV-vis of 5 shell CdSe/CdS QDs in toluene, with annealing time dependence; B: PL of 5 shell CdSe/CdS QDs in toluene, with annealing time dependence. Ex. wav. 400 nm.

1.3 The Dependence of G-Value on Heating Duration of 5th Monolayer Shell of CdSe/CdS QDs.

Measured in the excitonic region. Cysteine concentration is 13 mg/mL.

A. Before heating at 250 °C

B. After 15 mins

C. After 30 mins

D. After 1 hour
1.4 Quenching of 5 Shell CdSe/CdS QDs with Ibuprofen (100 mg/mL)

A: PL of 5 shell CdSe/CdS QDs, 1 hour after addition of drugs. Ex. wav. 400 nm; B: Quenching curve of 5 shell CdSe/CdS QDs, 1 hour after addition of drugs.

1.5 Quenching of 5 Shell CdSe/CdS QDs with Ibuprofen (500 mg/mL)

Experiment I

A: PL of 5 shell CdSe/CdS QDs, 21 hours after addition of drugs. Ex. wav. 400 nm; B: Quenching curve of 5 shell CdSe/CdS QDs, 21 hours after addition of drugs.
Experiment II

A: PL of 5 shell CdSe/CdS QDs, 1 hour after addition of drugs. Ex. wav. 400 nm; B: Quenching curve of 5 shell CdSe/CdS QDs, 1 hour after addition of drugs.

A. PL of 5 shell CdSe/CdS QDs, 18 hours after addition of drugs (not mixed before measuring.) Ex. wav. 400 nm. B. Quenching curve of 5 shell CdSe/CdS QDs, 18 hours after addition of drugs. (not mixed)
A: PL of 5 shell CdSe/CdS QDs, 19 hours after addition of drugs (mixed before measuring.) Ex. wav. 400 nm.; B: Quenching curve of 5 shell CdSe/CdS QDs, 19 hour after addition of drugs. (mixed)

A: UV-Vis of 5 shell CdSe/CdS QDs, 1 hour after addition of drugs; B: UV-Vis of 5 shell CdSe/CdS QDs 18 hours after addition of drugs (not mixed); C: UV-Vis of 5 shell CdSe/CdS QDs, 19 hours after addition of drugs (mixed).
1.6 DLS Spectra of L-/D-cys ZnS:Mn QDs in Absence and Presence of Oleylamine and BSA

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1.7 Additional UV-Vis and PL Spectra of Cadmium-based and Zinc-based QDs after PT

A: UV-Vis of 1-thio-β-D-glucose 5 shell CdSe/CdS QDs, in DMSO; B: PL of 1-thio-β-D-glucose 5 shell CdSe/CdS QDs, in DMSO. Ex. wav. 400 nm.

A: UV-Vis of L-/D-cysteine ZnS:Mn QDs with and without oleylamine, in water; B: PL of L-/D-cysteine ZnS:Mn QDs with and without oleylamine, in water. Ex. wav. 300 nm.

A: UV-Vis of 1-thio-β-D-glucose ZnS:Mn QDs, in DMSO; B: PL of 1-thio-β-D-glucose ZnS:Mn QDs, in DMSO. Ex. wav. 300 nm.
1.8 Publications relating to this work

1. **Article**: Enantioselective cytotoxicity of ZnS:Mn quantum dots in A549 cells  
   **Article DOI**: X  
   **Journal**: Chirality *(Accepted for publication, May 2017)*

*Note: Author name and institution are included in article; thus DOI is not included to maintain anonymity. Further information on article is available upon request.*