Dyes and Pigments 185 (2021) 108960

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: http://www.elsevier.com/locate/dyepig

Green synthesis of amino acid functionalized CuInS/ZnS- mTHPP conjugate for biolabeling application

Ncediwe Tsolekile [a,b,c], Sundararajan Parani [a,b], Rodney Maluleke [a,b], Olivier Joubert [d], Mangaka C. Matoetoe [c], Sandile P. Songca [e], Oluwatobi S. Oluwafemi [a,b,*]

[a] Department of Chemical Sciences (formerly Applied Chemistry), University of Johannesburg, P.O. Box 17011, Doornfontein, 2028, Johannesburg, South Africa
[b] Centre for Nanomaterials Science Research, University of Johannesburg, Johannesburg, South Africa
[c] Department of Chemistry, Cape Peninsula University of Technology, P.O. Box 652, Cape Town, 2000, South Africa
[d] Université De Lorraine, Faculté de Pharmacie, F-54001, Nancy Cedex, France
[e] Department of Chemistry, University of KwaZulu-Natal, Private Bag X 54001, Durban, 4000, South Africa

ARTICLE INFO

Keywords:
Functionalization
CuInS/ZnS
Leukemia (THP-1) cell line
Arginine
Bio-labeling
mTHPP

ABSTRACT

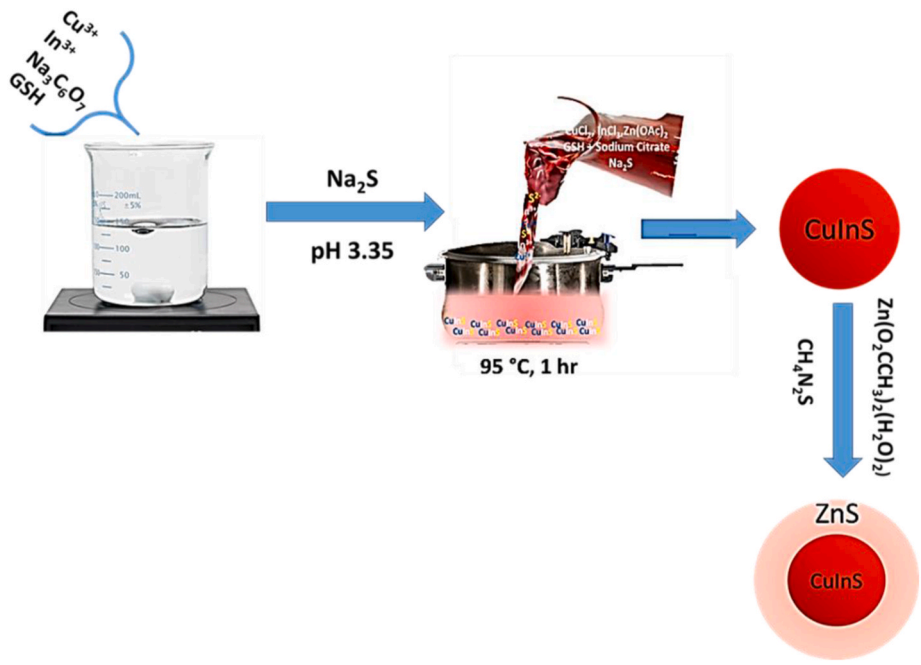
Quantum dots (QDs) have been resourceful in bio-applications such as fluorescent tagging, sensing, and bio-assays. Their bio-recognition and subsequent acceptance as foreign bodies by biomolecules largely depend on the surface structure of the QDs. Surface functionalization is thus a critical factor in their synthesis and design. In this work, we report on the surface modification of CuInS/ZnS QDs by an α-amino acid which plays an essential role in cell division and wound healing. The CuInS/ZnS QDs was synthesized via one-step eco-friendly method in a pressure cooker. To improve the biocompatibility and stability, the purified QDs were functionalized with L-arginine using carbodiimide chemistry to produce arginine (Arg) functionalized CuInS/ZnS QDs. Interest in the use of L-arginine amino acid in this study is a result of its zwitterion state, physiological properties, and most importantly its backbone structure which enables ease of conjugation. The Arg-CuInS/ZnS QDs were then conjugated to 5, 10, 15, 20-meso (hydroxyphenyl) porphyrin (mTHPP), a second-generation photosensitizing drug to improve the absorption efficiency of the porphyrin and tagging of cancer cells. This study examined for the first time the effect of amino acid functionalization on the luminescent, absorption and morphology properties of ternary CuInS/ZnS QDs and its porphyrin conjugate using various optical analyses. Furthermore, this study investigated the bio-labeling properties of the prepared material and tested this against leukemia (THP-1) cancer cell lines. The as-synthesized water-soluble biocompatible Arg-CuInS/ZnS - mTHPP conjugate proved to be useful for surface functionalization and as a possible bio-labeling agent.

1. Introduction

Quantum dots (QDs) are semiconductor materials with optoelectronic properties that can be tuned *in-situ* for diverse applications in fields such as computing, photo-catalysis, bio-imaging, and microbiology.[1–3] To improve their cellular uptake, intracellular localization, and other salient features (i.e. resistance to photo-bleaching, good stability and biocompatibility), QDs are often functionalized with targeting molecules such as; amino acids, aptamers and peptides.[4,5] Amino acids such as arginine have gained a lot of interest in surface modification due to the presence of bio-functional groups such as –COOH and -NH_2.[8,9] Moreover, they are easily recognized by the body's bio-components which eliminates their rejection by the body and are commercially available.[6] Surface functionalization of quantum dots is mostly achieved via covalent coupling strategies such as crosslinking (whereby two or more molecules are covalently bonded to improve on the desired properties of the materials) or via ligand exchange (where the hydrophobic layer of the organic solvent maybe replaced by bio-functional molecules).[7] The latter method possesses several disadvantages such as the reduction in photoluminescence intensity which reduces the quality of the functionalized material whereas crosslinking has proved to increase water solubility, aid conjugation (due to availability of binding groups such as amines, carboxyls etc.) and improve on the biological compatibility of the material.

* Corresponding author. Department of Chemical Sciences (formerly Applied Chemistry), University of Johannesburg, P.O. Box 17011, Doornfontein, 2028, Johannesburg, South Africa.
E-mail address: oluwafemi.oluwatobi@gmail.com (O.S. Oluwafemi).

https://doi.org/10.1016/j.dyepig.2020.108960
Received 13 May 2020; Received in revised form 21 October 2020; Accepted 22 October 2020
Available online 26 October 2020
0143-7208/



Scheme 1. Schematic diagram for the synthesis of ternary CuInS and CuInS/ZnS QDs.

To date, most quantum dot - amino acid functionalization has been performed on group II-VI (e.g. CdSe, ZnS etc.) and group IV-VI (e.g. PbTe, PbS) elements.[10–12] Noting the presence of toxic elements such as Cd and Pb in these reported studies, the amino acid-functionalized QDs have been rendered futile for biological applications. In this study, L-arginine was used as a surface modifying agent to improve on the biocompatibility of the ternary CuInS/ZnS QDs. L-arginine is α-amino acid which plays an essential role in the regulation of blood pressure amongst other biological functions. The interest in L-arginine as surface modifier is a result of its zwitterion state, physiological properties (which improves biocompatibility), and most importantly its highly active sites (–COOH and NH_2 structural backbone) which enables ease of conjugation.[13,14] CuInS/ZnS QDs is one of the most researched ternary QDs for their low toxicity, tunable emission spectra, large Stokes shift, and plasmonic properties which advantages it over cadmium based conventional binary QDs.[15] However, the synthesis of ternary QDs is still underdeveloped with reports mostly based on organic synthesis which are not suitable for biological applications. Moreover, most commonly used conventional methods (e.g. reflux) produce significantly low quantities of the QDs which is not viable for large scale production.

Porphyrins are macrocyclic photosensitizing drugs that have a high affinity for cancer cells. They are known for their photo-physical properties which allow for their use in cancer treatments such as photodynamic therapy. Porphyrins have wide absorption properties from ultraviolet–visible (UV–Vis) to near-infrared (NIR) regions which is highly attractive for biological applications.[16] However, their application has been hindered by their hydrophobic nature which also leads to aggregation and hence reduced their ability to produce singlet oxygen needed for tumor destruction. One way of solving this problem is by conjugation of porphyrin to nanomaterials such as QDs. The conjugation of QDs to porphyrins produces water-soluble biological systems with tunable emissions of the QDs and treatment efficacy of porphyrins. Furthermore, the conjugation of QDs to porphyrin improves the tagging of cancer cells compared to QDs alone.[17] In this work, we conjugated CuInS/ZnS QDs to 5, 10, 15, 20-meso (hydroxyphenyl) porphyrin (mTHPP). mTHPP is the parent porphyrin of clinically approved photosensitizing drug Temoporfin (brand name Foscan).[18] Its skeletal structure allows for further modification moreover, its high absorption coefficient and easily tunable absorption properties allows for its application in near-infrared regions where tissue is transparent.

Briefly, we report on the large-scale synthesis of ternary CuInS/ZnS QDs using a commercial kitchen pressure cooker. The pressure cooker method is advantageous over other conventional syntheses for large scale production. We were able to generate ~3.2 g of CuInS/ZnS QDs compared to <1 g with conventional syntheses such as reflux, autoclave methods. The prepared QDs were modified with L-arginine (Arg) amino acid using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a crosslinking agent. Following the Arg functionalization, this study examined for the first time the effect of amino acid functionalization on optical properties of ternary CuInS/ZnS QDs and its porphyrin conjugate. Furthermore, this study investigated the bio-labeling properties of the prepared material and tested this against leukemia (THP-1) cancer cell lines. The obtained fluorescent imaging suggested that the conjugate (Arg-CuInS/ZnS – mTHPP) can be used as a labeling agent.

2. Experimental

2.1. Chemicals

(Copper chloride ($CuCl_2$), indium chloride ($InCl_3$), sodium citrate dihydrate ($Na_3C_6H_5O_7$. $2H_2O$), L-glutathione (GSH), sodium sulphide (Na_2S), zinc acetate dihydrate ($Zn(O_2CCH_3)_2(H_2O)_2$), thiourea (CH_4N_2S), sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol (CH_3CH_2OH), L-arginine ($C_6H_{14}N_4O_2$), *N*,*N*-Dicyclohexylcarbodiimide (DCC)) were purchased from Sigma Aldrich, South Africa and used as received. Deionized water was used for synthesis and analysis.

2.2. Synthesis of ternary and quaternary quantum dots

2.2.1. Synthesis of CuInS/ZnS QDs

CuInS and CuInS/ZnS QDs were synthesized hydrothermally using a commercial kitchen pressure cooker based on our recently reported method with modifications .[19] In a typical reaction, $CuCl_2$ (0.213 g, 1.264 mmol), $InCl_3$ (1.106 g, 5 mmol), $Na_3C_6H_5O_7$ (5.88 g, 20.135 mmol), GSH (0.276 g, 0.898 mmol) were added to 2 L of deionized water under magnetic stirring. This was followed by the addition of Na_2S stock solution (1.95 g/50 mL, 25.00 mmol) under magnetic stirring to initiate

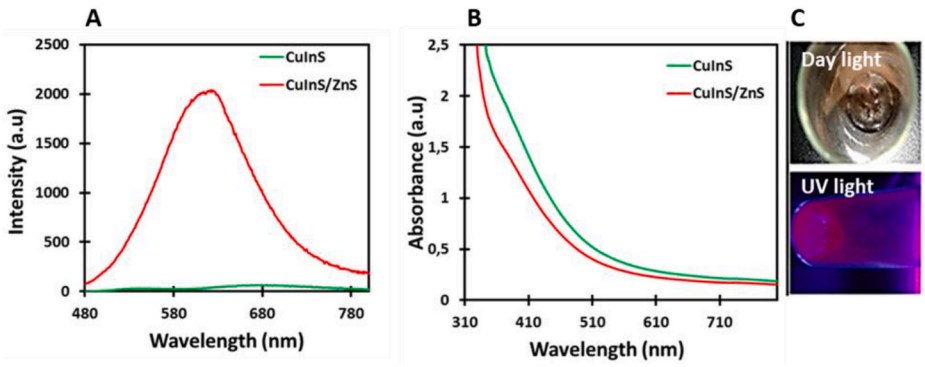


Fig. 1. (A) Photoluminescence and (B) absorption spectra of CuInS and CuInS/ZnS QDs (C) Digital photographs of CuInS/ZnS QD powders under day light and UV-light.

the reaction. The solution was heated at 95 °C for 1 h to produce CuInS at Cu:In (1:4) ratio in a commercial kitchen pressure cooker. After 1 h, the pressure cooker was cooled to (~86 °C), then $Zn(O_2CCH_3)_2(H_2O)_2$ (3.52 g/100 mL, 16.0 mmol) and CH_4N_2S (1.22 g/100 mL, 16.02 mmol) were added as ZnS precursors. The reaction was further heated in the commercial kitchen pressure cooker for 1 h at 95 °C to form CuInS/ZnS core/shell (Scheme 1).

2.2.2. Purification of the as-synthesized quantum dots

Following the synthesis, the QDs were subjected to purification via precipitation and decantation. This was performed to remove any reaction by-products and unreacted precursors. Ethanol was used for the precipitation. In a typical precipitation protocol, ethanol was added to the as-synthesized quantum dots at a ratio of 1: 3 (QDs solution: Ethanol). The solution of QDs in ethanol was covered and left to stand overnight to allow for sufficient precipitation. To further remove any unreacted precursors, the precipitate was centrifuged at 16 000 rpm for 5 min. This was repeated three times while washing with ethanol. The purified QDs were then allowed to dry at room temperature, evaporating the ethanol subsequently forming powdered QDs.

2.3. Functionalization of quantum dots with L-Arginine

Arginine functionalization was achieved using the method of Ncapayi et al., 2017 [20] with slight modifications. The arginine cross-linked GSH-capped QDs were obtained by dissolving 5 mg of CuInS/ZnS QDs in 10 mL DMSO. N, *N′*-Dicyclohexylcarbodiimide (DCC) (0.2 mmol) was added, followed by arginine (0.1 mmol) under vigorous stirring for 2.5 h. The arginine coated QDs were then precipitated and washed using ethanol as described above. The precipitate was re-dispersed in 10 mL water and the solution was sonicated. The solution was further filtered using a syringe filter with a nominal pore diameter of 0.45 μm.

2.4. Synthesis of 5,10,15,20-meso (hydroxyphenyl) porphyrin (mTHPP)

The synthesis of mTHPP was carried out according to Tsolekile et al., 2018 [21] with slight modifications. In a typical reaction, 3-hydroxybenzaldehyde (2.50 g, 20.47 mmol) was added to propionic acid (15 mL) in a 100 mL round-bottom flask equipped with a magnetic stirring bar and refluxed for 15 min at 140 °C. Distilled pyrrole (1.42 mL, 20.469 mmol) was then added quickly to the refluxing mixture and refluxed for an additional 1.5 h. After the reflux, excess propionic acid was evaporated at 220 °C. The material was cooled to room temperature and neutralized with 5% $NaHCO_3$. The precipitated crude porphyrin was washed with chloroform and finally chromatographed on silica gel column with ethyl acetate: n-hexane (2:1, v/v) mixture as eluent.

2.5. Bio-conjugation of Arg-CuInS/ZnS QDs with mTHPP

Arg-CuInS/ZnS (3 mL of a 2.5 mg/10 mL H_2O solution) was added to 1 mL of mTHPP (0.1 mg/10 mL CH_3OH) followed by mechanical stirring for 1 h. Conjugation of the Arg-CuInS/ZnS QDs to the free porphyrin was achieved via an esterification reaction between the hydroxyl groups of the free porphyrin and the hydrogen from the carboxylic group of the arginine functionalized QDs. The conjugate was precipitated out of the solution with ethanol and centrifuged to remove unconjugated Arg-CuInS/ZnS QDs or mTHPP.

2.6. Characterization

The photoluminescence spectra of the synthesized material were recorded using Shimadzu photoluminescence (PL), RF-6000 spectrophotometer equipped with spectro-flourometer laser light as an excitation source. The absorption properties of the as-synthesized materials were analyzed in the range of 200–900 nm using Lambda 25 Ultraviolet–Visible (UV–Vis), spectrometer. The surface chemistry of the purified and dried material was carried out by using PerkinElmer, Spectrum two UATR spectrometer at a frequency range from 400 to 4000 cm^{-1}. Transmission electron microscopy (TEM) was performed by using JEOL (JEM- 2100) transmission electron microscope, operating at 200 kV. X-ray diffraction (XRD) patterns of the as-synthesized QDs were collected by PANalyticalX'Pert x-ray diffractometer using a monochromatic Cu Kα radiation (λ = 0.15406 nm) at room temperature. Nuclear Magnetic Resonance (NMR) was analyzed using 500 MHz Bruker spectrometer using DMSO~d_6 solvent for background. The ^1H NMR samples were prepared by placing a concentrated solution (using DMSO~d_6) of the material in ^1H NMR tube and analyzed at room temperature.

2.7. Bio-imaging of as-synthesized material on leukemia (THP-1) cell line

For the bio-imaging studies, THP-1 cells (5×10^5 cells/well) were seeded into a 96-well plate. After 24 h, the media were replaced with 100 μl of media (DMEM 0% FBS) without phenol red to minimize interactions. The cells were exposed to 63.0 μg/mL of Arg-CuInS/ZnS QDs, mTHPP and Arg-CuInS/ZnS-mTHPP conjugate. After 24 h incubation, confocal images of treated THP-1 cancer cell line were examined against untreated cell control.

3. Results and discussion

Green synthetic approaches are reported as advantageous for the synthesis of QDs as they are cost-effective and eco-friendly. However, reported studies have only used harsh conditions such as high temperatures and organic reagents which are undesirable for large and industrial scale production.[22,23] This study reports the large-scale

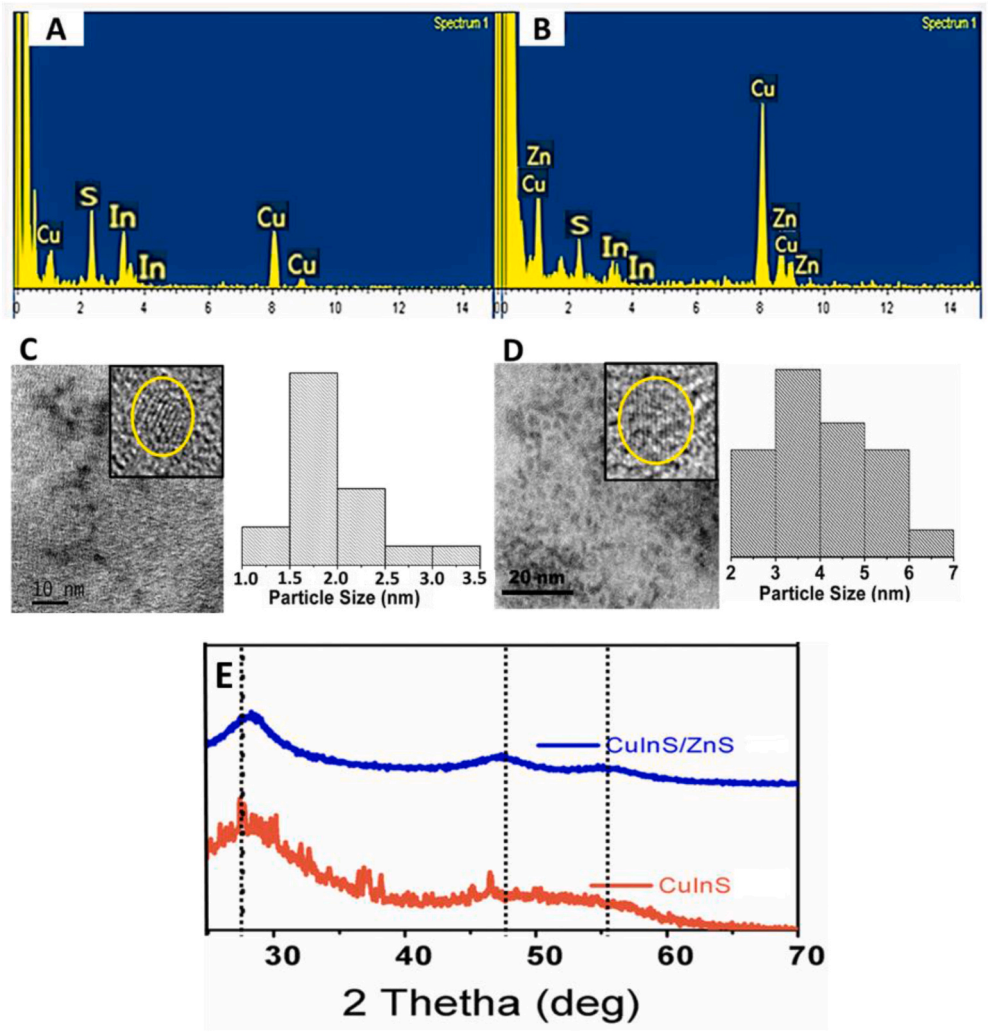


Fig. 2. (A–B) EDS of CuInS QDs and CuInS/ZnS QDs respectively and (C) TEM images of CuInS QDs, (D) CuInS/ZnS QDs and (E) XRD patterns of CuInS and CuInS/ZnS QDs.

synthesis of ternary CuInS/ZnS QDs using water as a solvent and an electrical pressure cooker as a reaction vessel. Scheme 1 depicts the experimental procedure for the synthesis of the water-soluble glutathione capped CuInS/ZnS QDs. In our experiment, glutathione and sodium citrate were used as stabilizing agents to balance the reactivity's of Cu^{+} as a soft acid and In^{3+} as a hard acid. To improve the photoluminescence of the CuInS QDs and eliminate any surface defects and dangling bonds on the CuInS surface, the CuInS was passivated with ZnS to form highly fluorescent CuInS/ZnS QDs. ZnS was selected as passivating agent due to its wide band-gap (~3.7 eV). Moreover, ZnS exhibits minimum lattice mismatch with $CuInS_2$ (~2.2%).[24]

Fig. 1 Shows the optical properties of CuInS and CuInS/ZnS QDs. As shown in the PL results, the CuInS core exhibited broad PL emissions with two peaks at 537 nm and 675 nm. The two emission peaks were attributed to two dissimilar trap surface states and intrinsic trap states typically observed in ternary QDs.[25–27] Following successful synthesis of the CuInS core, Zn and S precursors were added *in-situ*. This resulted in a huge increase in the PL intensity with its maximum blue-shifted from 675 to 623 nm (Fig. 1A). This increase in intensity was attributed to the elimination of some of the trap states and the blue-shift could be attributed to the diffusion of Zn^{2+} to the core CuInS with partial cation (Cu^{2+}, In^{3+}, and Zn^{2+}) exchange in-line with reported studies.[17,25] The absorption spectra of QDs (Fig. 1B) revealed broad bands with no distinct absorption, in-line with the reported studies.[28,29] The photoluminescence quantum yield of the core was measured to be 0.15% which increased to 4.9% after ZnS passivation. The digital photograph (Fig. 1C) displays the red fluorescence image of the CuInS/ZnS QDs in powdered form under the UV lamp (λ_{exc} = 352 nm) while the brown powder represents natural colour of powdered QDs. From the images, it can be deduced that the precipitation of QDs and subsequent formation of CuInS/ZnS powder does not affect the fluorescent nature of the material. Furthermore, it is worth mentioning that herein we were able to produce three times (~3.2 g) the quantity of QDs compared to our previously reported conventional synthetic method.[17,21]

Fig. 2A–B shows the EDS results of the CuInS and the CuInS/ZnS QDs respectively, the presence of Zn was observed in the CuInS/ZnS which was not seen in core CuInS. The morphology and average particle size of the as-synthesized CuInS core and CuInS/ZnS were estimated from the TEM micrograph. From the TEM (Fig. 2C–D), it can be observed that the particles of both the CuInS core and CuInS/ZnS shell are spherical. The average particle diameter, as determined from the TEM and particle size distribution curves is 1.75 nm for the CuInS QDs which increased to 3.5 nm after the ZnS shell passivation. The increase in size is attributed to the introduction of the shell over the core. The presence of lattice fridge confirmed the crystallinity in the synthesized CuInS/ZnS QDs. The XRD patterns (Fig. 2E) of the core CuInS QDs appears as a mixture of chalcopyrite and wurtzite phase as reported in the previous report by our group.[17] After shell passivation (CuInS/ZnS), the peaks shifted to higher angle which is ZnS (cubic zinc blende phase). The broad nature of

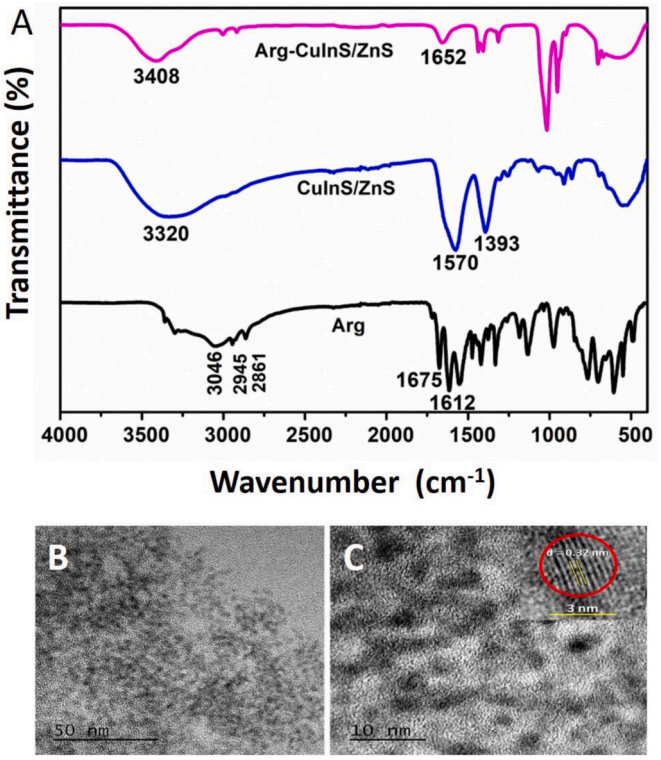


Fig. 3. (A) FTIR spectra of arginine, CuInS/ZnS QDs and Arg-CuInS/ZnS QDs. (B,C) TEM images of Arg-CuInS/ZnS. (C)Inset: HRTEM image of Arg-CuInS/ZnS QDs.

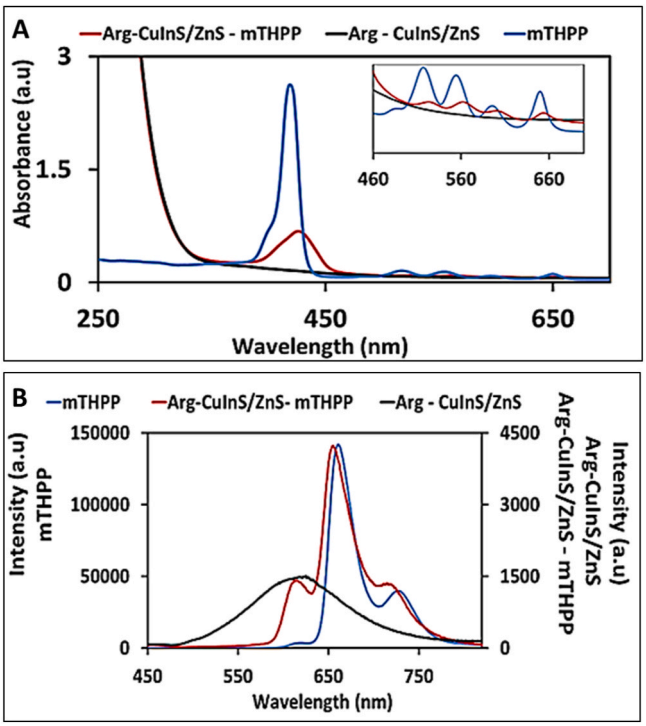


Fig. 4. (A) UV–Vis spectra of Arg-CuInS/ZnS QDs, mTHPP and Arg-CuInS/ZnS-mTHPP conjugates (insert: magnified view in the 460–700 nm wavelength range) and (B) PL spectra of Arg-CuInS/ZnS QDs, mTHPP and Arg-CuInS/ZnS-mTHPP conjugates.

the diffraction pattern is attributed to the nanocrystalline nature of the material as observed in the TEM.

3.1. Characterization of 5, 10, 15, 20-meso (hydroxyphenyl) porphyrin (mTHPP)

Fig. S1 shows the ^{1}H NMR spectra of mTHPP in DMSO. From the spectrum, the five expected environments of the porphyrin are present. The de-shielded singlet chemical shifts of 9.97 and 8.86 ppm were assigned to –OH and β-pyrrole respectively. The chemical shifts at 7.98–8.00 ppm and 7.19–7.21 ppm with 8.25 and 8.35 protons respectively were also assigned to the Ar–H while the shifts at 2.6 and 3.5 were attributed to the presence of phenyl groups. Due to the anisotropic effect of the ring system of mTHPP, the inner protons of pyrrole gave a low chemical shift at −2.89 ppm. The presence of this signal as a singlet suggests the inner protons are not coupled (at least within three bonds away) with no neighbouring protons. This was used as confirmation that a free base porphyrin had been synthesized in line with reported studies. [30] Analysis of the purified powdered mTHPP using FTIR is shown in S2. The cyclic structure of mTHPP consists of four phenolic rings and four interconnected pyrrole subunits which are connected by methylene bridges.[31] FTIR analysis of mTHPP characterized by stretching bands due the presence of O–H, *N*–H pyrrole and C═C of phenyl peaks. ^{1}H NMR spectroscopy supported by FTIR confirmed that pure mTHPP was successfully synthesized.

3.2. Functionalization of CuInS/ZnS QDs with arginine

CuInS/ZnS QDs was functionalized with arginine using DCC as a cross-linker. The functionalization proceeded via the formation of a DCC-CuInS/ZnS reactive intermediate. The exposure of the intermediate to arginine resulted in arginine functionalized CuInS/ZnS QDs. Excess or unreacted arginine/DCC was removed by centrifugation. FTIR analysis (Fig. 3A) of the CuInS/ZnS QDs, Arg-CuInS/ZnS QDs, and arginine was performed on the powdered samples. The spectrum of arginine shows its characteristic peak due to *N*–H stretching at 3055 cm^{-1}, C–H asymmetric and symmetric stretching at 2945 and 2842 cm^{-1} respectively and *N*–H bending at 1675 cm^{-1} and C═O stretching at 1612 cm^{-1}. The CuInS/ZnS QDs exhibited peaks attributed to O–H stretching at 3320 cm^{-1}, COO asymmetric and symmetric stretching at 1570 cm^{-1} and 1387 cm^{-1} respectively from the surface capping group GSH. After functionalization of QDs with arginine, it has been noted in the spectrum of Arg-CuInS/ZnS QDs that COO peaks of GSH disappeared and the new peak formed at 1652 cm^{-1} which indicated the formation of amide bond between carboxylic group of QDs surface GSH and amine group of Arg. These results confirmed the successful functionalization of the QDs with Arg. Fig. 3B and C depict the TEM monographs of the arginine functionalized CuInS/ZnS QDs. Surface functionalization with amino acid did not induce any aggregation, and the particles maintained their spherical and mono-dispersed nature. The lattice fringes in the Arg-CuInS/ZnS QDs suggested that the crystallinity was not affected by the functionalization.

3.3. Bio-conjugation of Arg-CuInS/ZnS - 5, 10, 15, 20-meso (hydroxyphenyl) porphyrin (mTHPP)

Fig. 4A represents the absorption spectral overlay of the Arg-CuInS/ZnS QDs, mTHPP and Arg-CuInS/ZnS QDs-mTHPP conjugate. The mTHPP porphyrin showed features of a free base porphyrin with the presence of all four Q-bands confirming that a free base mTHPP porphyrin was synthesized. The spectra show that the conjugate preserved the spectroscopic properties of both Arg-CuInS/ZnS QDs and mTHPP. Notable is the reduction in the absorbance and the redshift in

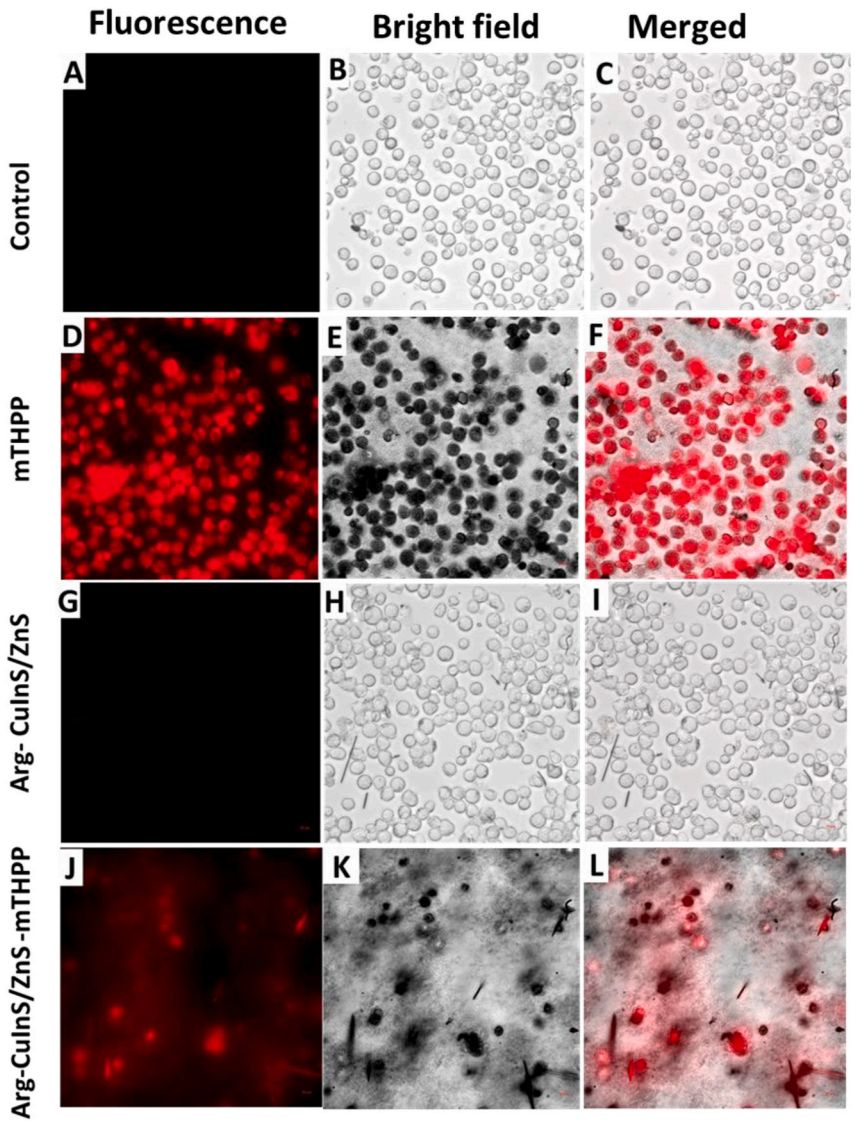


Fig. 5. Optical imaging of mTHPP, Arg-CuInS/ZnS QDs and Arg-CuInS/ZnS-mTHPP conjugate with respect to control.

the Q bands in the conjugate (insert). From Fig. 4B, it can be observed that mTHPP reveals two major emission peaks at 663 and 732 nm which are attributed to the emission from the T (0,0) and T (1,0) triplet states respectively and a weak shoulder peak at 618 nm which could be attributed the emission from the S (1,0) singlet state.[32] After the conjugation with QDs, it was noted that the intensity of the triplet emission decreased, however, the singlet emission increased with the slight blue-shift in peak positions. The decrease in intensity and shift in both absorption spectra of the conjugate relative to the freebase porphyrin could be attributed to the differences between the average dielectric constant of the mTHPP in solution as compared to that near the surface of the QDs. Moreover, the emission of QDs is completely quenched which indicated the possible fluorescence energy transfer (FRET) from QDs to the porphyrin.[33] The increase in the singlet emission suggested the interference of QDs in the intersystem crossing of the excited porphyrin molecule from singlet state to the triplet state. Nonetheless, further studies involving the investigation of photophysical transitions, surface analysis and mass spectra analysis are required to clearly understand the whole process.

3.4. Bio-imaging of mTHPP, Arg-CuInS/ZnS QDs and Arg-CuInS/ZnS-mTHPP conjugate on leukemia (THP-1) cell lines

The particle size of QDs determine their surface to volume ratio, this is known to significantly influence the material uptake and bio-distribution. While the shape of the particles affects cellular uptake, preferential interaction is critically affected by surface coating.[34] Our results shows that CuInS/ZnS QDs have been coated successfully with biocompatible arginine which did not significantly affect the properties of the material. Considering the above properties and photosensitizing capabilities of mTHPP, the bio-labeling capabilities of mTHPP, Arg-CuInS/ZnS QDs and Arg-CuInS/ZnS QDs -mTHPP conjugate was examined on leukemia (THP-1) cancer cells. The THP-1 cells were exposed to 65 μl of mTHPP, Arg-CuInS/ZnS QDs and Arg-CuInS/ZnS QDs-mTHPP conjugate as shown in Fig. 5. Following the incubation of THP-1 cells with the as-prepared material, the cell morphology of the THP-1 cells remained unaltered. Fig. 5 A, D, G and J shows the fluorescence images of the cell while parts B, E, H and K shows the bright field images. The merged images (C, F, I and L) depicted bright red fluorescence for the mTHPP and Arg-CuInS/ZnS-mTHPP conjugate as observed in the fluorescence images indicating the localization of the material and their entry into the THP-1 cells. From the confocal imaging, it can be concluded that the as-prepared material successfully imaged

the cells and demonstrated their potential use in bio-imaging and drug delivery.

4. Conclusions

Herein, novel arginine functionalized CuInS/ZnS QDs conjugated to porphyrin has been prepared and characterized. Briefly, water soluble, fluorescent CuInS/ZnS QDs were synthesized using a commercial kitchen pressure cooker with good yield. The synthesized QDs exhibited broad PL emissions and UV absorptions typical of chalcopyrite QDs. The surface of the CuInS/ZnS QDs was modified by functionalization with bio-compatible zwitterion L-arginine amino acid. The Arg-CuInS/ZnS QDs maintained PL peak position and crystallinity of the bare QDs. The functionalized Arg-CuInS/ZnS QDs was then conjugated with the porphyrin (mTHPP) and the results showed possible FRET between QDs and the porphyrin and the interference of QDs in the intersystem crossing of the porphyrin. Exposure of leukemia (THP-1) cell lines to the as-prepared material suggested that there was cellular interaction of the material with the biological environment of the cell. This is evident in the visible intercellular red fluorescence observed in the cells which is not observed in the control. The as-synthesized Arg-CuInS/ZnS- mTHPP conjugate material has cellular fluorescent labeling abilities and can be used for drug delivery and therapy applications.

Author contributions

Ncediwe Tsolekile- Investigation; Methodology, Validation, Writing - original draft.

Sundararajan Parani – Formal analysis, Writing - Review & Editing.

Rodney Maluleke – Formal analysis.

Olivier Joubert – Formal analysis (all biological work), Writing - Review & Editing.

Mangaka C Matoetoe -Conceptualization, Writing - Review & Editing.

Sandile P Songca -Conceptualization, Writing - Review & Editing.

Oluwatobi S Oluwafemi - Conceptualization, Methodology, Resources, Supervision, Project administrations, Funding acquisition and Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors will like to thank National Research Foundation, South Africa under the Nanotechnology Flagship Programme (Grant no: 97983), Competitive Programme for Rated Researchers (Grant no: 106060), Thuthuka (Grant no: 107295 and Grant No: 121986), Equipment-related travel and training (Grant no 109892), Cape Peninsula University of Technology and the University of Johannesburg, South Africa, Faculty of Science Research Committee, and University Research Committee, South Africa for financial support. We also thank Dr. Ikeoluwa A Oluwafemi for editing and grammatical correction and Ms Hendriette van der Walt (Mintek, SA) for the cell viability.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2020.108960.

References

[1] Nguyen AVT, Dao TD, T TT, Choi D, Park SYH, Yeo S. Sensitive detection of influenza a virus based on a CdSe/CdS/ZnS quantum dot-linked rapid fluorescent immunochromatographic test. Biosens Bioelectron 2020;155:112090.
[2] Wu T, Liu X, Liu Y, Cheng M, Liu Z, Zeng G, Shao B, Liang Q, Zhang W, He Q, Zhang W. Application of QD-MOF composites for photocatalysis: energy production and environmental remediation. Coord Chem Rev 2020;403:213097.
[3] Kloeffel C, Loss D. Prospects for Spin-Based quantum dots. Condens Matter Phys 2013;4:51–81.
[4] Liu J, Zhao X, Xu H, Wang Z, Dai Z. Amino acid-capped water-soluble near-infrared region $CuInS_2$/ZnS quantum dots for selective cadmium ion determination and multicolor cell imaging. Anal Chem 2019;14:8987–93.
[5] Arshad A, Akram R, Iqbal S, Batool F, Iqbal B, Khalid B, Khan AU. Aqueous synthesis of tunable fluorescent, semiconductor $CuInS_2$ quantum dots for bio-imaging. Arabian J.Chem 2019;12:4840–7.
[6] Pereira G, Monteiro CAP, Albuquerque GM, Pereira MIA, Cabrera MP, Filho PEC, Pereira GAL, Fontesa A, Santos BS. (Bio) conjugation strategies applied to fluorescent semiconductor quantum dots. J Braz Chem Soc 2019;30:2536–60.
[7] David NL, Michael CM. Principles of biochemistry. fourth ed. New York: W. H. Freeman; 2005, ISBN 0-7167-4339-6.
[8] Siddiq AM, Thangam R, Madhan B, Alam S. Green (gemini) surfactant mediated gold nanoparticles green synthesis: effect on triple negative breast cancer cells. Nano-Structures & Nano-Objects 2019;19:100373.
[9] Probst CE, Zrazhevskiy P, Bagalkot V, Gao X. Quantum dots as a platform for nanoparticle drug delivery vehicle design. Adv Drug Deliv Rev 2013;5:703–18.
[10] Masteri-Farahani M, Mollatayefeh N. Chiral colloidal CdSe quantum dots functionalized with cysteine molecules: new optical nanosensor for selective detection and measurement of morphine. Colloid Surface Physicochem Eng Aspect 2019;569:78–84.
[11] Kanagasubbulakshmi S, Gowtham I, Kadirvelu K, Archana K. Biocompatible methionine-capped CdS/ZnS quantum dots for live cell nucleus imaging. MRS Commun 2019;9:344–51.
[12] Li J, Mao M, Wu F, Li Q, Wei L, Ma L. Amino-functionalized CdSe/ZnS quantum dot-based lateral flow immunoassay for sensitive detection of aflatoxin B1. Analytical Methods 2018;10:3582–8.
[13] Sun M, Yang Li, Jose P, Wang L, Zweit J. Functionalization of quantum dots with multidentate zwitterionic ligands: impact on cellular interactions and cytotoxicity. J Mater Chem B 2013;1:6137–46.
[14] Vermaa N, Singh AK, Singh M. L-arginine biosensors: a comprehensive review. Biochem. Biophy. Reports 2017;12:228–39.
[15] Fuhr AS, Alexandrova AN, Sautet P. Stoichiometry-controllable optical defects in Cu_x in $_{2-x}S_y$ quantum dots for energy harvesting. J Mater Chem 2020;8:12556–65.
[16] Yang L, Zhou J, Wang Z, Li H, Wang K, Liu H, Wu F. Biocompatible conjugated porphyrin nanoparticles with photodynamic/photothermal performances in cancer therapy. Dyes Pigments 2020;182:108664.
[17] Tsolekile N, Nahle S, Zikalala N, Parani S, Sakho EHM, Joubert O, Matoetoe MC, Songca SP, Oluwafemi OS. Cytotoxicity, fluorescence tagging and gene-expression study of CuInS/ZnS QDS - meso(hydroxyphenyl) porphyrin conjugate against human monocytic leukemia cells. Sci Rep 2020;10:4936.
[18] Senge MO. mTHPC – a drug on its way from second to third generation photosensitizer? Photodiagn. Photodyn. Ther 2012;9:170–9.
[19] Tsolekile N, Ncapayi V, Obiyenwa GK, Matoetoe M, Songca S, Oluwafemi OS. Synthesis of meso-tetra-(4-sulfonatophenyl) porphyrin ($TPPS_4$) – CuInS/ZnS quantum dots conjugate as an improved photosensitizer. Int J Nanomed 2019;14:7065–78.
[20] Ncapayi V, Parani S, Songca SP, Kodama T, Oluwafemi OS. Simple green synthesis of amino acid functionalized CdTe/CdSe/ZnSe core-multi shell with improved cell viability for cellular imaging. Mater Lett 2017;189:168–71.
[21] Tsolekile N, Ncapayi V, Parani S, Sakho EHM, Matoetoe MC, Songca SP, Oluwafemi OS. Synthesis of fluorescent $CuInS_2$/ZnS quantum dots—porphyrin conjugates for photodynamic therapy. MRS Commun 2018;8:398–403.
[22] Krishnaswamy S, Panigrahi P, Kumaar S, Nagarajan GS. Effect of conducting polymer on photoluminescence quenching of green synthesized ZnO thin film and its photocatalytic properties. Nano-Structures & Nano-Objects 2020;22:100446.
[23] Chen C, Wu D, Chan Yu, Lin CC, Chung P. Evaluations of the chemical stability and cytotoxicity of CuInS2 and CuInS2/ZnS core/shell quantum dots. J Phys Chem C 2015;119:2852–60.
[24] Akkerman QA, Genovese A, George C, Prato M, Moreels I, Casu A, Marras S, Curcio A, Scarpellini A, Pellegrino T, Manna L, Lesnyak V. From binary Cu_2S to ternary Cu-In-S and quaternary Cu-In-Zn-S nanocrystals with tunable. ACS Nano 2015;9:521–31.
[25] Zhang B, Yucheng W, Chengbin Y, Siyi H, YuanYiping G, Yue Z, Hilmi WVD, Liwei L, Yong K. The Composition effect on the optical properties of aqueous synthesized Cu–In–S and Zn–Cu–In–S quantum dot nanocrystals. Phys Chem Chem Phys 2015;17:25133–41.
[26] Chen Y, Li S, Huang L, Pan D. Low-cost and gram-scale synthesis of water soluble Cu–In–S/ZnS core/shell quantum dots in an electric pressure cooker. Nanoscale 2014;6:1295–8.
[27] Deng D, Qu L, Cheng Z, Achilefu S, Gu Y. Highly luminescent water-soluble quaternary Zn – Ag – in – S quantum dots and their unique precursor S/in ratio-dependent spectral shifts. J Lumin 2014;146:364–70.
[28] Zou W, Li L, Chen Y, Chen T, Yang Z, Wang J, Liu D, Lin G, Wang X. In vivo toxicity evaluation of PEGylated $CuInS_2$/ZnS quantum dots in BALB/c mice. Front Pharmacol 2019;10:437.

[29] Jindal S, Giripunje SM, Kondawar SB, Koinkar P. Green synthesis of $CuInS_2$/ZnS core-shell quantum dots by facile solvothermal route with enhanced optical properties. J Phys Chem Solid 2018;14:163–72.
[30] Rojkiewicz M, Kuś P, Kozub P, Kempa M. The synthesis of new potential photosensitizers [1]. Part 2. Tetrakis- (hydroxyphenyl) porphyrins with long alkyl chain in the molecule. Dyes Pigments 2013;99:627–35.
[31] Wong W, Rawn CJ. Crystallography of functional materials. Crystals 2017;7:279.
[32] Feng Y, Liu L, Hu S, Liu Y, Ren Y, Zhang X. Förster resonance energy transfer properties of a new type of near-infrared excitation PDT photosensitizer: CuInS2/ZnS quantum dots-5-aminolevulinic acid conjugates. RSC Adv 2016;6:55568–76.
[33] Lemon CM, Karnas E, Bawendi MG, Nocera DG. Two-photon oxygen sensing with quantum dot-porphyrin, conjugates. Inorg Chem 2013;52:10394–406.
[34] Ankamwar Balaprasad. Size and shape effect on biomedical applications of nanomaterials. Biomedical Engineering – Technical Applications in Medicine 2012: 93–114.