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# L-Cysteine-capped CdTe Quantum Dots as a Fluorescence Probe for Determination of Cardiolipin

**Wenfeng ZHAO,*† Yingsing FUNG,* Waisum O,** and M. P. L. CHEUNG****

**Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P. R. China*
***Department of Anatomy, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P. R. China*

This paper described the investigation of surface-modified quantum dots (QDs) as a fluorescence probe for the detection of cardiolipin. A single-step method for preparation of non-toxic and photo-stable cadmium telluride (CdTe) QDs capped by L-cysteine in aqueous solution was developed. The prepared QDs were characterized by high-resolution transmission electron microscopy, X-ray diffraction spectrometry, Fourier transform infrared spectrometry and spectrofluorometry. These functional QDs were used as a fluorescence probe for cardiolipin determination based on the fluorescence quenching. The optimum fluorescence intensity was found to be at pH 7.4 with QDs concentration of $4 \times 10^{-5}$ mol $L^{-1}$. The effect of other phospholipids on the intensity of CdTe QDs showed a low interference response. Under optimized conditions, the quenched fluorescence intensity was linear with the concentration of cardiolipin in the range of $1.33 \times 10^{-7}$ – $10.4 \times 10^{-7}$ mol $L^{-1}$ ($r = 0.9976$) and a detection limit ($S/N = 3$) of 18.5 nmol $L^{-1}$. The proposed method was applied to the determination of cardiolipin content of HepG2 cell samples before and after oxidative stress with satisfactory results.

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## Introduction

Cardiolipin is a unique phospholipid that is found in the membranes of bacteria and mitochondria.[1,2] It has been found to play an important role in all basic mitochondrial functions, including energy transformation, electron transfer, apoptosis, and membrane integrity.[3,4] Alterations in the content of cardiolipin have been associated with mitochondrial dysfunction in multiple tissues in a variety of pathological conditions, including ischemia, hypothyroidism, aging, and heart failure.[5] Therefore, the measurement of cardiolipin could lead to a greater understanding of certain diseases at a molecular level. Due to the lack of a selective chromophore in the structure, several strategies involving the specific interaction of cardiolipin with the fluorescence probe 10-*N*-nonyl acridine orange (NAO), and its analogue compounds, have been developed for quantitative determination.[6-8] However, these methods suffered from drawbacks of lack of simplicity, sensitivity or practicability. Thus, research on the utilization of cardiolipin is still limited. A very efficient and rapid method for the determination of cardiolipin is needed.

Quantum dots (QDs) are very attractive fluorescent probes for biomedical applications and have shown great promise as potential replacements for organic fluorescent dyes.[9,10] Compared with traditional organic fluorescence probes, QDs possess several qualities that make them very advantageous for fluorescent tagging, such as broad excitation wavelength range, narrow emission spectrum, size-tunable emission peak, longer fluorescence lifetime and negligible photobleaching.[11-13] Moreover, procedures for preparation of organic fluorescence probes are usually complex, time-consuming and labor-intensive. As important semiconductor nanoparticles, much attention has been focused on cadmium telluride (CdTe) because of its relatively high optical absorption coefficient, and its advanced photovoltaic properties.[14,15]

To broaden its applicability in new areas, we explored a method based on the fluorescent quenching of CdTe QDs by cardiolipin. In this study, we synthesized L-cysteine-capped CdTe QDs in aqueous solution through a straight forward, one-pot process by using low-cost inorganic salts and a low-toxicity stabilizer. L-Cysteine was used as the stabilizer because it could provide thiol groups to bond to the CdTe QDs surface and make them available for interaction with target materials.[16] With the interaction condition and style studied in detail, cardiolipin content in the stressed HepG2 cells could be evaluated as compared to control. We found this method to be sensitive, rapid, accurate and simple, and provided a new and reliable means for the quantitative determination of cardiolipin.

## Experimental

*Reagents and chemicals*

All chemicals were used without further purification. Doubly deionized water (DDW, 18.2 MΩ $cm^{-1}$) was obtained from a WaterPro water purification system (Labconco Corp., Kansas City, MO). $CdCl_2 \cdot 2.5H_2O$ (Analytical Reagent), tellurium powder (Te) (99%), L-cysteine (99%) and sodium borohydride ($NaBH_4$) (98%) were purchased from Sigma-Aldrich Chemical Co. Cardiolipin (heart, bovine, $F_W = 1493.895$) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Other phospholipids, including L-$\alpha$-phosphatidylcholine (egg, chicken, $F_W = 770.123$, PC), L-$\alpha$-phosphatidylethanolamine (egg, chicken, $F_W = 744.034$, PE), L-$\alpha$-phosphatidylinositol (liver,

† To whom correspondence should be addressed.
E-mail: zhaowf@hkusua.hku.hk

bovine, $F_W$ = 909.110, PI), and L-$\alpha$-phosphatidylserine (brain, porcine, $F_W$ = 812.041, PS) were also purchased from Avanti Polar Lipids Inc. Stock solutions of phospholipids were prepared in ethanol at a concentration of 100 µmol $L^{-1}$ and were further diluted with DDW whenever necessary. A Tris–HCl buffer solution (0.1 mol $L^{-1}$ Tris, pH 7.4) was used in the experiments.

*Apparatus*

All fluorescence measurements were performed with a Perkin-Elmer Model LS-50B luminescence spectrometer (Perkin-Elmer, USA), with a 1-cm quartz cell. Fourier transform infrared (FT-IR) spectra were recorded with a BIO-RAD FTS 165 spectrophotometer (Hercules, CA) using KBr matrix. High-resolution transmission electron microscopy (HRTEM) images and the selected-area electron diffraction (SAED) patterns of the QDs were acquired on a Philips microscope Tecnai 20 (Philips, Eindhoven, Holland) operated at an acceleration voltage of 200 kV. X-Ray powder diffraction (XRD) spectra were taken on a Bruker D8 Advance X-ray diffractometer (Bruker, Karlsruhe, Germany) with Cu $K_\alpha$ radiation ($\lambda$ = 1.5406 Å).

*Synthesis of L-cysteine-capped CdTe QDs*

The procedures for the preparation of L-cysteine-capped CdTe QDs were as described by Gaponik[17] with some modifications. NaHTe solution was used for Te source instead of the $H_2Te$ gas reported by Gaponik *et al*. The direct injection of NaHTe was easier, allowed more control of amounts and more reproducible ways to produce high-quality CdTe QDs. NaHTe solution was prepared *via* the reaction of Te powder with excess $NaBH_4$ at a molar ratio of 1:20. Briefly, 0.3026 g $NaBH_4$ was transferred into a small flask and 4 mL of DDW was added. Afterwards, 0.0510 g of Te powder was added to the flask, and the reacting system was stirred under a $N_2$ atmosphere. During the reaction, a small outlet connected to the flask was kept open to discharge the pressure from the $N_2$ and the resulting $H_2$. Thus NaHTe solution was obtained as the color of the solution changed from black to white. Then 400 µL of the freshly prepared NaHTe solution was injected into an oxygen-free $CdCl_2$ (0.04 mol $L^{-1}$) solution containing L-cysteine stabilizer (0.01 mol $L^{-1}$) at pH 11.2 under steam of $N_2$. The final volume of the CdTe dispersion was 50 mL. The molar ratio for Cd:Te:L-cysteine was 1:0.2:2.5. The solution was stirred and refluxed for 1 h at 96°C. A colloidal solution with a CdTe concentration of 0.008 mol $L^{-1}$ was prepared (referred to as $Cd^{2+}$). The quantum yield of 12.5% was measured for the prepared CdTe QDs at room temperature by comparing with the fluorescence emission of Rhodaminutese 6G.[18]

*Procedures for spectrofluorometric determination of cardiolipin*

To a series of 10 mL calibrated test tubes, certain amounts of CdTe QDs solution, 1.0 mL of Tris–HCl buffer solution (pH 7.4) and an appropriate volume of cardiolipin stock solution were added sequentially. The mixture was then diluted to volume with DDW and mixed thoroughly. The fluorescence intensity of the solution was recorded at 535 nm with an excitation wavelength of 330 nm. The excitation and emission slit widths were 10 nm.

*Mitochondria membrane preparation from HepG2 cells and lipid extraction*

HepG2 cells were cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM) with 10% fetal bovine serum (FBS) and 100 U/mL penicillin. The cells were cultured in flasks for 3 days prior to experiments. Then the cells were dissociated from the culture flasks with 0.25% trypsin in PBS, resuspended in DMEM containing 10% FBS, and counted with a hemocytometer. Finally, 5 mL of cell suspension ($4 \times 10^6$ cells/mL) was homogenized on ice using a glass homogenizer (Kontes, Düsseldorf, Germany). The cells were then centrifuged for 5 min at 1000*g* at 4°C to remove the unbroken cells. The supernatant was then centrifuged for 20 min at 12000*g* at 4°C to obtain the pellet (the membrane fraction).

A modified Folch method[19] was used to extract the lipids. Briefly, the harvested membrane fraction was suspended in 1 mL of 0.01 mol $L^{-1}$ Tris–HCl buffer (pH 7.4). The membrane fraction was placed in a conical tube and 3.75 mL 1:2 (v/v) $CHCl_3$:MeOH was added; the solution was then vortex mixed carefully. To the same tube, 1.25 mL $CHCl_3$ was added and vortexed well. Finally, 1.25 mL DDW was added and again the mixture was vortexed carefully. After centrifugation at 7000*g* for 15 min, the bottom phase (chloroform layer) was withdrawn carefully by means of a pipette. The chloroform phase was evaporated under vacuum at 40°C until it was dry. Then the tube was placed in the freezer until analysis.

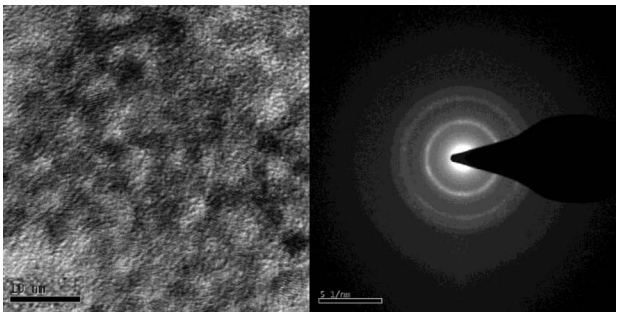

Fig. 1 HRTEM (left) and SAED pattern (right) of a representative of L-cysteine-capped CdTe QDs sample.

## Results and Discussion

*Characterization of the L-cysteine-capped CdTe QDs*

The morphology of the prepared CdTe QDs was visualized by HRTEM, as shown in Fig. 1 (left panel). Under HRTEM, the CdTe QDs appeared as spherical particles with average sizes of approximately 3 nm. On average, the size distribution was around 10% without any size-selective treatment. Distinct lattice fringes were observed indicating a crystalline product, with an approximate lattice fringe distance of $d$ = 3.0 Å; this was later confirmed by SAED (Fig. 1, right panel) and XRD (Fig. 2).

The corresponding SAED pattern shows distinguishable diffraction rings and is also indicative of crystallinity (Fig. 1, right panel). The XRD spectrum obtained from powdered precipitated fractions of L-cysteine-capped CdTe QDs shows peaks at 23.7°, 40.1°and 46.7° (Fig. 2), corresponding to the crystal planes 111, 220 and 311, confirming that it had a cubic zinc blend crystalline structure.[17]

Figure 3 shows the FT-IR spectra of free L-cysteine and L-cysteine-capped CdTe QDs. The presence of characteristic peaks around 1550 – 1600 $cm^{-1}$, and at 1400 $cm^{-1}$ correspond to the carboxyl group. The IR absorption bands observed around 2200 – 3200, 2500 – 2600 and 2100 $cm^{-1}$ are due to the stretching vibrations of the N–H of the $-NH_3$ group.

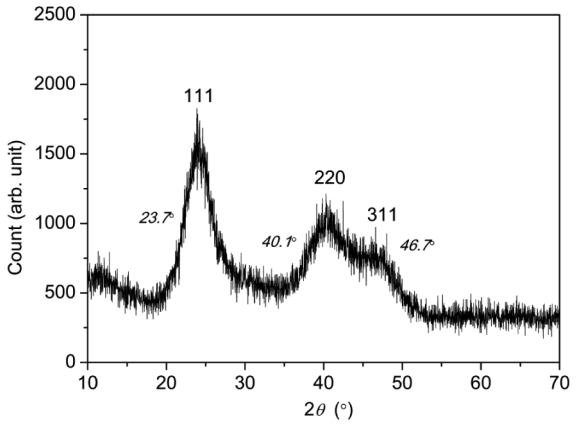


Fig. 2 Powder XRD spectra of the L-cysteine-capped CdTe QDs.

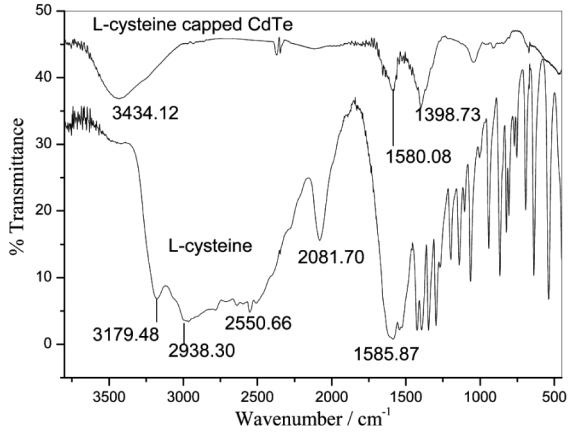


Fig. 3 FT-IR spectra of the L-cysteine and L-cysteine-capped CdTe QDs.

The observed features at 2550 – 2670 $cm^{-1}$ represent the thiol group.[20] Compared to the IR absorption spectra of free L-cysteine (Fig. 3, lower curve), only the characteristic peaks of the carboxyl and amino groups were observed on the L-cysteine-capped CdTe QDs (Fig. 3, upper curve). The peak around 3400 $cm^{-1}$ indicates the bending vibration of the N–H of the $-NH_2$ moiety on the L-cysteine-capped CdTe QDs. The bands of the $-NH_2$ group observed on the L-cysteine-capped CdTe QDs could be ascribed to the basification of the $NH_3^+$ group of L-cysteine during the synthesis. The covalent affinity of $Cd^{2+}$ for the –SH group of L-cysteine resulted in the disappearance of the thiol group vibration on the L-cysteine-capped CdTe QDs.

*Effect of the pH of L-cysteine-capped CdTe QDs-cardiolipin system*

It is well known that use of different buffer pH could lead to a drastic change of fluorescence intensity of QDs, which could affect the sensitivity and selectivity of target materials.[13] In order to develop a sensitive and rapid spectrophotometric method for determination of cardiolipin, we studied the effect of pH of the solution on the fluorescence intensity was studied and the results are shown in Fig. 4. Maximal and stable fluorescence intensity was obtained between 7.3 and 8.0 (relative fluorescence intensity in the absence and presence of cardiolipin). It could be

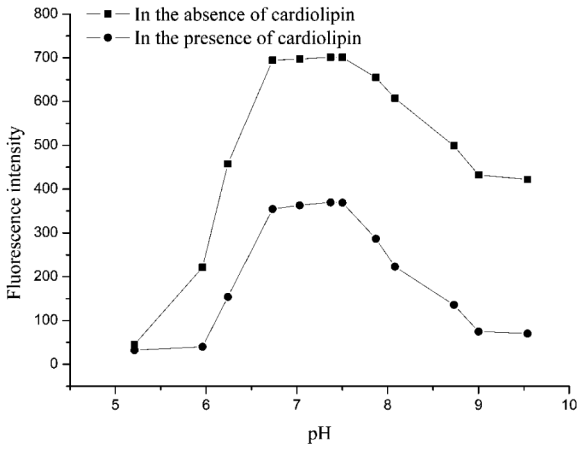


Fig. 4 pH dependent fluorescence intensity of the L-cysteine-capped CdTe QDs-cardiolipin system.

rationalized that during the preparation, cadmium and thiol were in excess, and the pH was adjusted to 11.2, so that cadmium-thiol complexes were formed in the solution. When the CdTe solution became acidic, partial thiols and cadmium ions would be released from the cadmium-thiol complexes, the particle surface covered with thiols increased. Therefore, the trap sites on the CdTe surface would be removed, dramatically improving the fluorescence intensity.[21] The low fluorescence intensity in acidic medium was the result of the dissociation of the $Cd^{2+}$-L-cysteine nanoparticles due to the protonation of the surface-binding thiols. At higher pH, the hydrolysis of cardiolipin may also decrease the fluorescence intensity.[22] Therefore, a pH of 7.4 with Tris–HCl as buffer solution (0.01 mol $L^{-1}$ Tris) was chosen to run the assay.

*Effect of the concentration of the L-cysteine-capped CdTe QDs*

The influence of the concentration of L-cysteine-capped CdTe QDs on the change in normalized fluorescence intensity was investigated with a constant concentration of cardiolipin ($5.2 \times 10^{-7}$ mol $L^{-1}$) at pH 7.4. When the concentration was too low, the limited QDs could not occupy all binding sites of cardiolipin existing in the system. When the concentration of QDs was too high, the fluorescence intensity may decrease because of the self-quenching effect. Considering the sensitivity and the linear range, we chose $4 \times 10^{-5}$ mol $L^{-1}$ as the concentration of CdTe QDs in this work.

*Stabilization of fluorescence intensity*

An initial experiment showed that the fluorescence intensity of the L-cysteine-capped CdTe-cardiolipin system reached equilibrium in 5 min and the fluorescence signals remained stable for more than 30 min. We recorded the fluorescence intensity after the system was incubated for 15 min.

*Fluorescence characteristic of the L-cysteine-capped CdTe-cardiolipin system*

The emission spectra of L-cysteine-capped CdTe QDs in the absence and presence of various concentrations of cardiolipin were recorded in the 0.01 mol $L^{-1}$ Tris–HCl (pH 7.4) buffer. As seen in Fig. 5, the emission maximum of L-cysteine-capped CdTe QDs was at 535 nm. The fluorescence intensity of L-cysteine-capped CdTe QDs at 535 nm decreased significantly with increasing cardiolipin concentrations. The quenching effect of cardiolipin on the fluorescence intensity of CdTe QDs

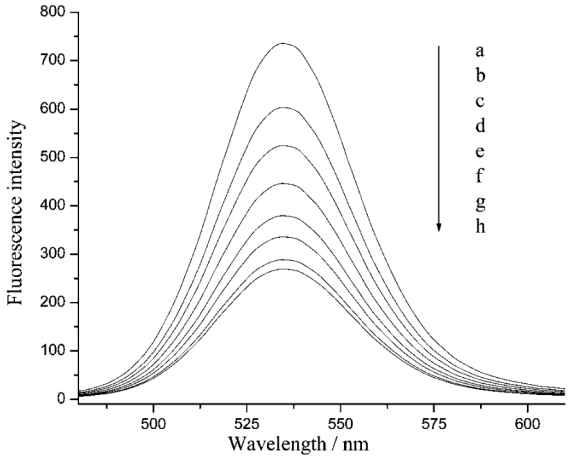


Fig. 5 Fluorescence spectra of L-cysteine-capped CdTe-cardiolipin system. (a) – (h), The emission spectra of L-cysteine-capped CdTe in the presence of cardiolipin at various concentrations ($\times 10^{-7}$ mol $L^{-1}$, pH 7.4): 0, 1.3, 2.6, 3.9, 5.2, 6.5, 7.8 and 9.1.

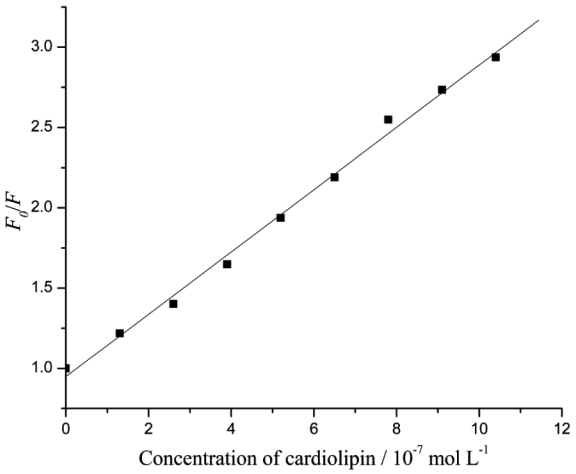


Fig. 6 Stern–Volmer plot of cardiolipin concentration dependence of the fluorescence intensity of L-cysteine-capped CdTe QDs with a Stern–Volmer constant $K_{sv}$ $1.78 \times 10^6$ $M^{-1}$.

was found to be concentration-dependent. Therefore, this system could be used for the development of a sensitive and selective probe for cardiolipin.

The spectral change was attributed to a combined action of hydrophobic and electrostatic interaction between QDs and cardiolipin. Cardiolipin is distinguished from other phospholipids by having two negative charges and by carrying four acyl groups.[2] It can bind protons at physiological pH; thus, the electrostatic interaction between the negatively charged lipid head groups of cardiolipin and the protonated amine moieties of L-cysteine-capped CdTe QDs played a crucial role in quenching effect. An initial electrostatic interaction appeared to be accompanied by deep penetration of QDs nanoparticles into the acyl chain region of cardiolipin resulting in a significant quenching effect. The experimental data confirmed the concept of an insertion of the QDs nanoparticles deep into the hydrophobic environment of the lipid acyl chains.[23]

Under optimal conditions, the quenching effect of cardiolipin on the fluorescence emission of L-cysteine-capped CdTe QDs was found to be concentration dependent, and was best described by a Stern–Volmer type equation:[24]

$$\frac{F_0}{F} = 1 + K_{sv}[CL]$$

where $F_0$ and $F$ are the fluorescence intensity in the absence and the presence of the quencher cardiolipin, respectively. [CL] is the concentration of cardiolipin; $K_{sv}$ is the Stern–Volmer quenching constant. As shown in Fig. 6, a good linear relationship between $F_0/F$ and the concentration of cardiolipin was observed in the range $1.33 \times 10^{-7}$ – $10.4 \times 10^{-7}$ mol $L^{-1}$ with a correlation coefficient of 0.9976. $K_{sv}$ was found to be $1.78 \times 10^6$ L $mol^{-1}$. The limit of detection, calculated following the $3\sigma$ IUPAC criteria, was 18.5 nmol $L^{-1}$. The relative standard deviation was 2.1%, on the basis of eleven replicate measurements of the quenched fluorescence intensity in the presence of $3.9 \times 10^{-7}$ mol $L^{-1}$ cardiolipin.

*Effect of foreign phospholipids*

Phospholipids (PLs) with a variety of functional groups including L-$\alpha$-phosphatidylcholine (PC), L-$\alpha$-phosphatidylethanolamine (PE), L-$\alpha$-phosphatidylinositol (PI) and

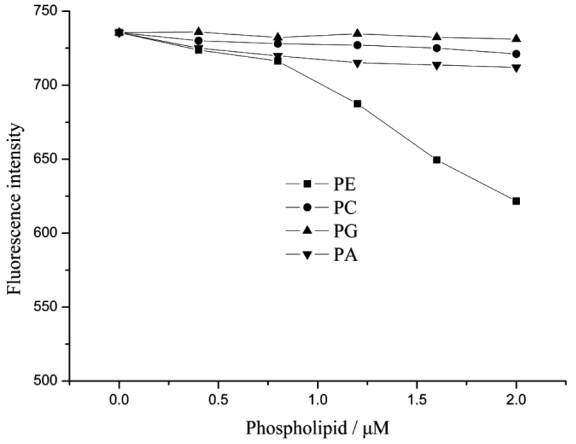


Fig. 7 Effect of foreign phospholipids on the fluorescence intensity of L-cysteine-capped CdTe QDs.

L-$\alpha$-phosphatidylserine (PS) were selected as potential interfering lipids. The PLs chosen here represented the most common lipid compositions of mitochondria. No significant fluorescence quenching of L-cysteine-capped CdTe QDs by other PLs was noted, only PI did appear to quench the fluorescence at concentrations above 8 μmol $L^{-1}$ (Fig. 7). In general, PL concentrations in biological samples would be expected to be less than this. The PL compositions of mitochondria found at significant levels are primarily phosphatidylcholine (40%) and phosphatidylethanolamine (35%),[25] neither of which was found to interfere with the fluorescence. The percentages of phosphatidylinositol (5%) and phosphatidylserine (1%) are markedly lower than cardiolipin (18%).[25] Under the conditions of sample analysis, we believe that foreign PLs, in particular PI, would not cause interference with the determination of cardiolpin.

*Comparison with other methods*

The linear range and detection limit of several selected fluorometric methods for cardiolipin are summarized in Table 1. It can be seen that organic dyes has a high concentration linear range. NAO and its analogue compounds are either expensive

Table 1 Comparison of the linear range and detection limit (LOD) of several selected fluorometric methods for determination of cardiolipin

| Reagent | ($\lambda_{ex}/\lambda_{em}$)/ nm | Linear range/ $10^{-7}$ mol $L^{-1}$ | LOD/ $10^{-9}$ mol $L^{-1}$ | Ref. |
|---|---|---|---|---|
| NAO[a] | 518/530 | 2 – 100 | 200 | 7 |
| C14-AO[b] | 498/521 | 0 – 25 | 70 | 8 |
| C18-AO[c] | 498/520 | 0 – 25 | 40 | 8 |
| L-Cysteine-capped CdTe | 330/535 | 1.3 – 10.4 | 18.5 | This work |

a. NAO: 10-*N*-nonyl acridine orange.
b. C14-AO: *n*-tetradecyl acridine orange.
c. C18-AO: *n*-octadecyl acridine orange.

Table 2 Results for the determination of cardiolipin in cell extract samples ($n$ = 3)

| Sample | HepG2(C)[b] | | HepG2(FCCP)[c] | |
|---|---|---|---|---|
| | [CL][a]/(μM) ± SD | Rec., % | [CL][a]/(μM) ± SD | Rec., % |
| 1 | 0.612 ± 0.21 | 89 | 0.304 ± 0.21 | 93 |
| 2 | 0.648 ± 0.17 | 92 | 0.332 ± 0.22 | 93 |
| 3 | 0.632 ± 0.17 | 92 | 0.321 ± 0.18 | 95 |

a. [CL]: concentration of cardiolipin.
b. HepG2(C): HepG2 cell without FCCP treatment as control.
c. HepG2(FCCP): HepG2 cells were incubated with 10 μM FCCP for 15 min.

or difficult to synthesize, demanding more than 24 h. However, the proposed method is much easier for preparation of the nanoparticles and offers a higher sensitivity.

*Analytical performance*

The proposed method was applied to the determination of cardiolipin content in HepG2 cells. The assay results are shown in Table 2. HepG2 cells incubated with FCCP (carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, a classical uncoupling agent) were induced to undergo apoptosis. Supporting the theory that there is a loss of cardiolipin from mitochondria under oxidative stress,[26] a significant decrease of cardiolipin content was found as compared to the control cells. These results were also in good agreement with a previous study.[27] It should be noted that the cardiolipin levels in the three samples showed small differences due to the variance of cell growth. Even so, it is evident the cardiolipin loss could be detected during FCCP-mediated apoptosis and it is likely that by increasing the number of cells used in these analyses, the variability in these measurements can be reduced. The accuracy of the method was investigated by recovery studies. When known amounts of cardiolipin ($2.6 \times 10^{-7}$ mol $L^{-1}$) were added to a previously prepared sample, 89 – 95% recoveries were obtained. This indicated that the developed method was accurate, sensitive and reproducible.

## Conclusions

L-Cysteine-capped CdTe QDs were synthesized in aqueous solution through a straightforward, one-pot process by using safe and low-cost inorganic salts as precursors. A new method was proposed for the determination of cardiolipin based on the fluorescence quenching of L-cysteine-capped CdTe QDs and with a detection limit of 18.5 nmol $L^{-1}$. In addition to sensitivity, other advantages of this method include its simplicity and rapidity. The results suggested that L-cysteine-capped CdTe QDs may provide a new class of fluorescence probe for use in chemical sensing and biotechnology applications.

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