Colloidal Synthesis of MoS$_2$ Quantum Dots: Size-dependent Tunable Photoluminescence and Bioimaging

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Although the synthesis of two-dimensional (2D) layered MoS$_2$ nanomaterials have been developing rapidly, there are many technical issues in preparing MoS$_2$ quantum dots (QDs) with photoluminescence property. Herein, we design a facile colloidal chemical route to prepare photoluminescent MoS$_2$ QDs using the ammonium tetrathiomolybdate [(NH$_4$)$_2$MoS$_4$] as precursors and oleyl amine as reducing agent. The optical property and structure of as-prepared MoS$_2$ QDs are investigated systematically. Resultant MoS$_2$ QDs exhibit fluorescence ($\lambda_{\text{max}}$=575 nm; quantum yield, 4.4%), spherical morphology with uniform thickness of ~3 nm and excitation-dependent PL phenomenon. Moreover, resultant MoS$_2$ QDs show size-dependent tunable photoluminescence in wide visible region. With the help of the amphiphilic compound, resultant MoS$_2$ QDs could be transferred from organic to aqueous phase. MoS$_2$ QDs in aqueous solution have many advantages, such as good dispersion, low toxicity and photoluminescent property which make them possess promising applications in optoelectronic and biological fields. In this study, the 293T cells are used as a model to evaluate the fluorescence imaging of MoS$_2$ QDs. The results confirm fluorescent signal appears in cytoplasm which demonstrates as-prepared MoS$_2$ QDs could be used as a probe for real-time optical cellular imaging.

1. Introduction

In recent decades, two-dimensional (2D) layered nanomaterials such as graphene have aroused increasing research interest in industrial and scientific fields because of their unique properties and wide potential applications.\textsuperscript{1-3} Among the numerous kinds of 2D layered nanomaterials, much attention has been devoted to the molybdenum disulfide (MoS$_2$) due to its capacity to form monolayers.\textsuperscript{4, 5} MoS$_2$, with graphene-analogous structure, composed of Mo atoms sandwiched between two layers of hexagonally close packed sulfur atoms, possesses many excellent properties and could be widely applied in many fields such as in catalysts, hydrogen storage, solid superlubricants, and solid-state secondary lithium battery.\textsuperscript{6-10} Current researches focus on the electrical and catalytic properties of MoS$_2$ nanosheets.\textsuperscript{11, 12} Several recent researches have shown that MoS$_2$ enable an indirect-to-direct bandgap transition due to the hybridization between Pz orbitals of S atoms and d orbitals of Mo atoms, when its thickness is thinned to monolayer.\textsuperscript{13} This transition results in the giant enhancement of its photoluminescence (PL) efficiency in MoS$_2$ monolayers, which receives tremendous attention for many applications in optoelectronic and biological fields.\textsuperscript{14, 15} However, bulk MoS$_2$ is an indirect bandgap material which shows excellent chemical and thermal stability, the difficulties remain in preparation of photoluminescent MoS$_2$ nanomaterials.

To date, some efforts have been made to achieve the synthesis of photoluminescent MoS$_2$ materials and the existing approaches can be divided into three ways including exfoliation, substrate growth, and colloidal synthesis.\textsuperscript{16-20} For example, Coleman et al. proposed liquid-phase exfoliation of commercial MoS$_2$ powers in a suitable organic solvent with the aid of ultrasonication as a route to prepare photoluminescent MoS$_2$\textsuperscript{17} Sow and Duan et al. have synthesized photoluminescent MoS$_2$ films on various substrates using the chemical vapor deposition method.\textsuperscript{18, 19} However, the exfoliation methods or substrate growth methods had been allowed for the preparation of photoluminescent MoS$_2$ inherent disadvantages limited their further applications. For instance, it is hard to control their size distribution, shape, or nature of their edges in the exfoliation methods. Moreover, the substrate growth methods could create large monolayers with high crystallinity and control over shapes, but is intrinsically limited by the amount of monolayers being produced. Compared with them, the colloidal chemical synthesis strategy can in principle enable the preparation of large batches of MoS$_2$ monolayers with good crystallinity, monodispersity, and control over the edges.\textsuperscript{20} Indeed colloidal chemical route strategy has been used to synthesize Cd-based quantum dots (QDs) and rare-earth doped nanocrystals.\textsuperscript{21, 22} However, few literatures have reported the preparation of MoS$_2$ QDs with tunable PL through the colloidal chemical synthesis. On the other hand, despite the growing researches on the PL of MoS$_2$, there have been only a limited number of investigations about their biological applications.
In this work, a facile colloidal chemical route was designed to synthesize photoluminescent MoS$_2$ QDs using the ammonium tetrathiomolybdate ((NH$_4$)$_2$MoS$_4$) as precursors and oleyl amine (OLA) as reducing agent. OLA not only helped to form MoS$_2$ as reducing agent but also worked as stabilizer agents on the surface of MoS$_2$ QDs. Through controlling the amount of OLA, the optical property, size and morphology of product in the colloidal solution could be tunable. As-prepared MoS$_2$ QDs with the size at 4.5 nm displayed excitation dependent emission behavior. Moreover, resultant MoS$_2$ QDs showed tunable PL from blue to red due to increasing the size by changing the amount of OLA and reaction time. With the help of the cetyltrimethyl ammonium bromide (CTAB), the resultant MoS$_2$ QDs could be transferred from organic to aqueous phase. Formed CTAB–MoS$_2$ QDs had good dispersion, high toxicity and PL properties in aqueous solution which showed promising application in bioimaging.

2. Experimental

2.1 Materials

Oleic acid (OA, 90 %), 1-octadecene (ODE, 90 %) and oleyl amine (OLA, 70 %) were purchased from Sigma–Aldrich. Ammonium tetrathiomolybdate ((NH$_4$)$_2$MoS$_4$), cetyltrimethyl ammonium bromide (CTAB), chloroform, methanol, and acetone were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All the chemicals were used as received without purification. De-ionized water was used in all experiments.

2.2 Synthesis of MoS$_2$ quantum dots

MoS$_2$ quantum dots (QDs) were synthesized by a facile colloidal approach. In the typical process, a mixture of (NH$_4$)$_2$MoS$_4$ (0.05 mmol), OA (1 mL, 2.9 mmol), OLA (3 mL, 6.2 mmol), and ODE (6 mL, 17 mmol) was degassed at room temperature and heated up to 120 °C under vacuum for 2.5 h with stirring. The resulting homogeneous solution was switched to a dry nitrogen atmosphere, heated to 250 °C slowly and maintained at this temperature for 3 h. The temperature of the solution was allowed to cool under 100 °C. The resulting MoS$_2$ QDs were precipitated by addition of acetone, rinsed with methanol, and then redispersed in 10 mL of chloroform for further using. Using a similar method, other samples with various sizes were prepared under different experimental conditions.

2.3 Phase transfer of hydrophobic MoS$_2$ quantum dots to aqueous solution

The hydrophobic MoS$_2$ QDs could be further functionalized by various cationic surfactants such as CTAB through the ligand wrapped reaction, enabling the phase transfer of MoS$_2$ QDs from hydrophobic to hydrophilic media. In a typical process, 2 mL aforementioned MoS$_2$ QDs (3.0 mg mL$^{-1}$) in chloroform was added into 10 mL of CTAB solution (3.64 g L$^{-1}$). A subsequent ultra-sonication (~15 min) led to the stable white emulsion. With vigorous stirring at 50 °C for 24 h, a transparent solution was formed. After centrifugation to remove the free surfactants, the hydrophilic CTAB–MoS$_2$ QDs were collected by vacuum freeze-drying.

2.4 Cellular imaging

293T cells ($2^{10}$ cells/ml) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin using 96-well plate. Suspensions (20 µg/mL) of CTAB–MoS$_2$ QDs from the stock solution were prepared with Dulbecco’s phosphate buffer saline (DPBS). After sonication for 10 min to ensure complete dispersion, an aliquot (typically 0.01 mL) of the suspension was added to the well of a chamber slide containing the cells cultured for 4 h. The chamber slide was then incubated at 37 °C in a CO$_2$ incubator for 4 h for CTAB–MoS$_2$ QDs uptake (only 20 µg of MoS$_2$ QDs to 150 µL of culture medium (10$^5$ cells) was added). Prior to fixation of the cells on the slide for 65 inspection with a confocal fluorescence microscope, the excess CTAB–MoS$_2$ QDs were removed by washing 3 times with warm DPBS.

2.5 Characterization Methods

Photoluminescence experiments were performed with a Shimadzu RF–5301 PC spectrofluorimeter. X-ray photoelectron spectroscopy (XPS) using Mg $K_α$ radiation (1253.6 eV) was collected in a VG ESCALAB MKII spectrometer. Binding energy calibration was based on C 1s at 284.6 eV. X-ray diffraction (XRD) data was collected on a Siemens D–5005 X–ray diffractometer with Cu $K_α$ radiation ($λ = 1.5418Å$). The Fourier transform infrared spectroscopy (FTIR) was measured at wavenumbers ranging from 500 cm$^{-1}$ to 4000 cm$^{-1}$ using a Nicolet 6700 FTIR spectrophotometer. The morphology and mean diameter of QDs were characterized JEM–2100 transmission electron microscope (TEM) operating at 200 kV. Atomic force microscopy (AFM) measurements were made on NS3A Multimode Instrument (Jilin University, China). The confocal microscopy images were taken at Olympus Fluoview FV1000 (Jilin University, China). Raman measurements were carried out using an InVia laser micro-Raman spectrometer. All measurements were performed at room temperature under ambient conditions.

3. Results and discussion

3.1 The synthesis of MoS$_2$ quantum dots

The photoluminescent MoS$_2$ quantum dots (QDs) were prepared in the colloidal solution containing (NH$_4$)$_2$MoS$_4$ (0.05 mmol), OA (1 mL, 2.9 mmol), OLA (3 mL, 6.2 mmol), and ODE (6 mL, 17 mmol) under 250 °C for 3 hours. (NH$_4$)$_2$MoS$_4$ worked as Mo and S sources simultaneously which showed numerous applications in preparation of MoS$_2$ nanomaterials. As previous reports, OLA have the capacity to work as reducing agents and help to form lots of nanomaterials. In this colloidal solution, OLA could reduce (NH$_4$)$_2$MoS$_4$ to synthesize MoS$_2$ nanomaterials. Moreover, OLA and OA seem to be stabilizing reagents and they were beneficial to control the size and property of MoS$_2$. Then the optical properties of these MoS$_2$ QDs were investigated by photoluminescence (PL)
spectroscopy at room temperature. As shown in Fig. 1a, as-prepared MoS$_2$ QDs exhibited intense fluorescence with peak maximum at 575 nm under the excitation wavelength of 500 nm. The PL quantum yield of resultant MoS$_2$ QDs reached up to 4.4% using Rhodamine 6G (quantum yield, 0.95 in ethanol) as the standard, which was higher than that of MoS$_2$ QDs prepared by other methods.\textsuperscript{14,15,24}

Fig. 1. a, The photoluminescence spectrum of MoS$_2$ QDs in chloroform solution. The inset images show the photographs of MoS$_2$ QDs: b, in room light and c, under a UV light source emitting 365 nm light.

Moreover, resultant MoS$_2$ QDs had good dispersion in chloroform solution and there was no noticeable precipitation, which was attributed to OLA and OA as capping layer (Fig. 1b). Fig. 1c was an image of a cuvette under 365 nm radiation, the container of which the MoS$_2$ QDs were dispersed in chloroform. The strong gray fluorescence was an obvious concern due to the wide half maximum.

Transmission electron microscopy (TEM) revealed the as-prepared MoS$_2$ QDs to be 4.5 ± 0.5 nm in diameter (inset of Fig. 2a), with no large nanoparticles or aggregation (Fig. 2a).

Element mapping pattern of resultant QDs (Fig. 2c and 2d) contains two elements Mo and S. These results suggested the formation of small sized MoS$_2$ QDs in the colloidal solution. To further confirm the morphology and thickness of the as-formed MoS$_2$ QDs, atomic force microscopy (AFM) topography images of these nanostructures were analysed (Fig. 2e). The AFM image also supported the spherical morphology, with uniform thickness of ~3 nm (Fig. 2f).

The structure and composition of as-prepared MoS$_2$ QDs were investigated through X-ray diffraction (XRD), Raman measurements and X-ray photoelectron spectroscopy (XPS). As shown in Fig. 3a, the diffraction peaks match the standard peaks of hexagonal phase MoS$_2$ (a = b = 0.316 nm, c = 1.230 nm, JCPDS card no. 37-1492).\textsuperscript{25} The diffraction peak (~14.5°) of pure MoS$_2$ was not observed in the XRD. The spectrum of resultant QDs was recorded in a wet, paste-like form in order to avoid drying and stacking. Prior research indicated that the diffraction peak was corresponding to the c-plane of MoS$_2$ and could be used to study the structure of MoS$_2$, which was composed of Mo atoms coordinated with S atoms to form the S-Mo-S sandwich layer.\textsuperscript{26} The absence of this peak indicated that the quantum dots are few layers and too thin to be detected by XRD, which is well consistent with the AFM images and previous reports.\textsuperscript{12}

Fig. 2. a, Typical TEM image of as-prepared MoS$_2$ QDs (inset, size distribution: the average size was 4.5 ± 0.5 nm); b, high-resolution TEM image of as-prepared MoS$_2$ QDs; c and d EDX element mapping of MoS$_2$ QDs; e and f AFM image and height profile of the MoS$_2$ QDs.

3.2 The Characterization of MoS$_2$ QDs
The Raman spectrum of bulk MoS$_2$ material is well known with two main modes, out-of-plane $A_{1g}$ vibrations and in-plane $E_{1g}^{2g}$, located at $\approx$408 and $\approx$382 cm$^{-1}$, respectively.\textsuperscript{27} Compare with bulk MoS$_2$ material, we can see from Fig. 3b, (i) blue-shift for $E_{1g}^{2g}$ (384 cm$^{-1}$) and red-shift for $A_{1g}$ (406 cm$^{-1}$), (ii) the peak spacing between $E_{1g}^{2g}$ and $A_{1g}$ decreases and (iii) the intensities of $E_{1g}^{2g}$ and $A_{1g}$ reduces. The Raman spectrum of resultant MoS$_2$ QDs displayed two typical phonon modes of $E_{1g}^{2g}$ and $A_{1g}$ at 384 and 406 cm$^{-1}$, which further confirmed the formation of small QDs (Fig. 3b). The binding energies of Mo 3d$_{3/2}$, Mo 3d$_{5/2}$ peaks at 228.9 eV and 232.2 eV, S 2p$_{3/2}$ and S 2p$_{1/2}$ peaks at 162.5 eV and 163.6 eV indicated that Mo$^{4+}$ and S$^{2-}$ were the dominant oxidation states.\textsuperscript{28} The asymmetric peaks and tailing spectra (at about 236 eV) for Mo 3d$_{3/2}$ and Mo 3d$_{5/2}$ in the products were likely due to the existence of a small amount of Mo$^{6+}$.	extsuperscript{29} These results suggest the colloidal reduction approach is a facile way to prepare uniform MoS$_2$ QDs. Using the similar method, the photoluminescent WS$_2$ QDs were synthesized. Resultant WS$_2$ QDs had good dispersion in chloroform and exhibited blue fluorescence with $\lambda_{\text{max}}$ at 475 nm (Fig. S1).

### 3.3 Tunable Photoluminescence of MoS$_2$ QDs

It is well known that the excitation-dependent PL phenomenon has never been reported for metal-based semiconductor QDs, but it is the unique characteristic for carbon dots, graphene QDs and MoS$_2$ QDs.\textsuperscript{14,30-32} The PL properties of the prepared MoS$_2$ QDs investigated at different excitation wavelengths are presented in Fig. 4. It was observed that the increase in the excitation wavelength from 400 to 560 nm leads to a red shift in the PL spectra of the QDs over emission wavelengths ranging from 538 to 594 nm. The excitation dependent luminescence indicates polydispersity of the MoS$_2$ QDs dispersions, which is characteristic of colloidal synthesis method.\textsuperscript{14,22} This excitation-dependent PL behavior of fluorescent nanomaterials is useful in multicolor imaging applications.\textsuperscript{33} As reported in the literature, the luminescence properties of MoS$_2$ QDs depend on their particle size, which is similar as the quantum size effect of semiconductor QDs.\textsuperscript{14,15} This means that the aim to control the PL of MoS$_2$ QDs could be realized by adjusting their size.

![Fig. 4. The excitation dependent PL indicating the polydisperser nature of MoS$_2$ QDs.](Image)

In colloidal solution, some experiment conditions, including reaction temperature and time, the amount of ligands, would influence the size of nanomaterials. In this study, we found the ratios of OA to OLA and reaction time had the ability to change the PL property of the resultant MoS$_2$ QDs. As shown in Fig. 5a, with increasing the amount of OLA, the tunable PL emission was observed at room temperature and the spectra exhibited a significant red shift ($\sim$120 nm) from 430 nm to 550 nm. Meanwhile, the corresponding size of MoS$_2$ QDs increased from the 3.0 to 4.5 nm (Fig. S2). Besides, the size could increase from 2.0 nm to 7.0 nm by increasing the time from 0.5 to 8.0 h (Fig. S3). Accordingly, the fluorescence could shift from 430 nm to 610 nm (Fig. 5b).

### 3.4 The bioapplication of MoS$_2$ QDs

The extraordinary PL properties of MoS$_2$ QDs with smaller size make it have great potential in optoelectronic and biological applications.\textsuperscript{14,15} However, as-prepared MoS$_2$ QDs in the colloidal way could be only dispersed in organic solution (chloroform and toluene), which is not suitable for bioapplication. To solve this problem, the organic-soluble fluorescent nanomaterials should be transferred to aqueous phase as previous reports.\textsuperscript{34} With the help of amphiphilic compounds (CTAB and SDS), these MoS$_2$ QDs could be easily transferred from chloroform to aqueous phase. In aqueous solution, the PL of MoS$_2$ QDs was observed and no peak shift occurred (Fig. S4). Fourier transform infrared (FTIR) spectra (Fig. S5) were used to identify the surface functional groups present on the QDs’ surface in aqueous solution. The characteristic stretching bands were the same as functional...
Fig. 6. Viability of 293T cells after 24 h incubation with different concentrations of CTAB–MoS$_2$ QDs in the cell medium as determined by the MTT assay.

Fig. 7. (a) bright field, (b) confocal fluorescent and (c) overlay images of 293T cells incubating with CTAB-functionalized MoS$_2$ QDs for 24 h.

By taking these advantages of the as-prepared MoS$_2$ QDs, which have the properties of easy preparation, inherited florescence, and good biocompatibility, the cell internalization and intracellular distribution of resultant CTAB-functionalized MoS$_2$ QDs were evaluated by confocal laser fluorescence microscopy. Fig. 7a–c respectively revealed the bright field, confocal fluorescent and overlay images of 293T cells incubating with CTAB-functionalized MoS$_2$ QDs for 24 h. From the bright field image of 293T cells, we could see that the cells incubated with resultant MoS$_2$ QDs are still maintain their normal morphology, thus indicating the good biocompatibility of as-prepared MoS$_2$ QDs in this specific dose and time point. The fluorescent image irradiated by 350 nm, showed bright green fluorescence within the cells that indicated the uptake behaviors of 293T cells. It could be seen that most of fluorescent signal appeared in cytoplasm. This result demonstrated as-prepared MoS$_2$ QDs could be used as a probe for real-time optical cellular imaging.

Conclusion

In summary, the fluorescent MoS$_2$ QDs were synthesized by a facile colloidal method. Resultant QDs with uniform size exhibited intense fluorescence ($\lambda_{max}$=575 nm; quantum yield, 4.4%) and good dispersion in chloroform. The excitation dependent luminescence phenomenon was observed due to the polydispersity of MoS$_2$ QDs dispersions. The size of resultant MoS$_2$ QDs could be controlled according to the amount of oleyl amine or reaction time. The resultant MoS$_2$ QDs showed tunable luminescence in wide visible region from blue fluorescence (430 nm) to red fluorescence (600 nm) depending on increasing QDs size. With the help of cetyltrimethyethyl ammonium bromide (CTAB), the MoS$_2$ QDs could be easily transferred from chloroform to aqueous phase. In aqueous solution, CTAB-functionalized MoS$_2$ QDs showed photoluminescent property, good biocompatible and low toxicity. By the cell staining assay and the fluorescence microscope, we successfully demonstrate the employment of photoluminescent property of MoS$_2$ QDs with smaller size in cellular imaging. Furthermore, this synthesis might provide a versatile approach to synthesize QDs of transition metal dichalcogenides on a large scale.

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Notes and references


