CHAPTER 2

Experimental

2.1 Chemical reagents and equipments

2.1.1 Chemical reagents

1) Silver nitrate, $AgNO_3$, assay 99.8 %, RCI Labscan, Thailand.
2) Sodium hydroxide, NaOH, assay 97.0 %, RCI Labscan, Thailand.
3) Indium nitrate, $In(NO_3)_3$, assay 99.9 %, Aldrich, Germany.
4) Sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$, assay 99.5 %, CarloErba, Italy.
5) L-cysteine, $C_3H_7NO_2S$, assay 99.0 %, Merck, Germany.
6) Thioacetamide, C_2H_5NS, assay 99.0 %, Acros organics, Belgium.
7) Dimethyl sulfoxide, DMSO, Sigma-Aldrich, USA.
8) 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide, MTT, BIO BASIC, Canada.
9) RPMI1640, GIBCO, USA.
10) Trypsin-EDTA, GIBCO, USA.
11) Doxorubicin hydrochloride, DOX, FLUKA, Switzerland.
12) Carboxymethyl cellulose sodium salt, CMC, FLUKA, Switzerland.

2.1.2 Equipments

1) Hotplate and magnetic stirrer, Fisher Scienctific, USA.
2) Ultrasonic bath, Bandelin, Sonorex, Germany.
3) Analytical balance, Model PA-SERIES, Ohaus, USA.
4) Oven, Model UE-400, Memmert, Germany.
5) X-ray diffractometer, XRD, JDX-3530, JEOL, Japan.

6) X-ray photoelectron spectroscope, XPS, AXIS Ultra DLD, Kratos, USA.
7) Tranmission electron microscope, TEM, JEM-2010, JEOL, Japan.
8) Dynamic light scattering, DLS, Malvern Instruments Zetasizer Nano-S, UK.
9) Fourier transform infrared spectrometer, FTIR, Bruker Tensor 27, Germany.
10) Thermogravimetric analyzer, TGA, TGA-50 Analyzer, Shimadzu, Japan.
11) UV-Visible spectrophotometer, Lambda, Perkin Elmer, UK.
12) Photoluminescence, PL, LS 50B, Perkin Elmer, UK.
13) Fluorescence microscope, DMI 4000B, Leica, Singapore.
14) Flow cytometer, Becton Dickinson, Switzerland.

2.2 Synthesis of CMC-$AgInS_2$ QDs

1 mmol $AgNO_3$, 1 mmol $In(NO_3)_3$ and 0.1 g CMC were dissolved in 100 mL deionized water with continuous stirring. 2 M NaOH was dropped to adjust pH of the solution to 11. Then, 3 mL of 0.2 M C_2H_5NS was added drop-wise to the above solution. Similarly, $Na_2S_2O_3 \cdot 5H_2O$ or $C_3H_7NO_2S$ was used instead of C_2H_5NS. The final mixtures were put in Teflon-lined stainless autoclaves and treated at 200 °C for 2 and 24 h. The precipitates were obtained and washed with deionized water. Finally, the CMC-$AgInS_2$ QDs were re-dispersed in deionized water and kept at 4 °C for further studies. Details of the experiment are summarized in Table 2.1.

Table 2.1 Experimental details for the synthesis of CMC-$AgInS_2$ QDs.

Sample	$AgNO_3$ (mmol)	Sulfur source	Temperature (°C)	Time (h)
1	1	C_2H_5NS	200	2
2	2	C_2H_5NS	200	2
3	3	C_2H_5NS	200	2
4	1	C_2H_5NS	200	24
5	2	C_2H_5NS	200	24
6	3	C_2H_5NS	200	24
7	1	$Na_2S_2O_3 \cdot 5H_2O$	200	24
8	1	$C_3H_7NO_2S$	200	24

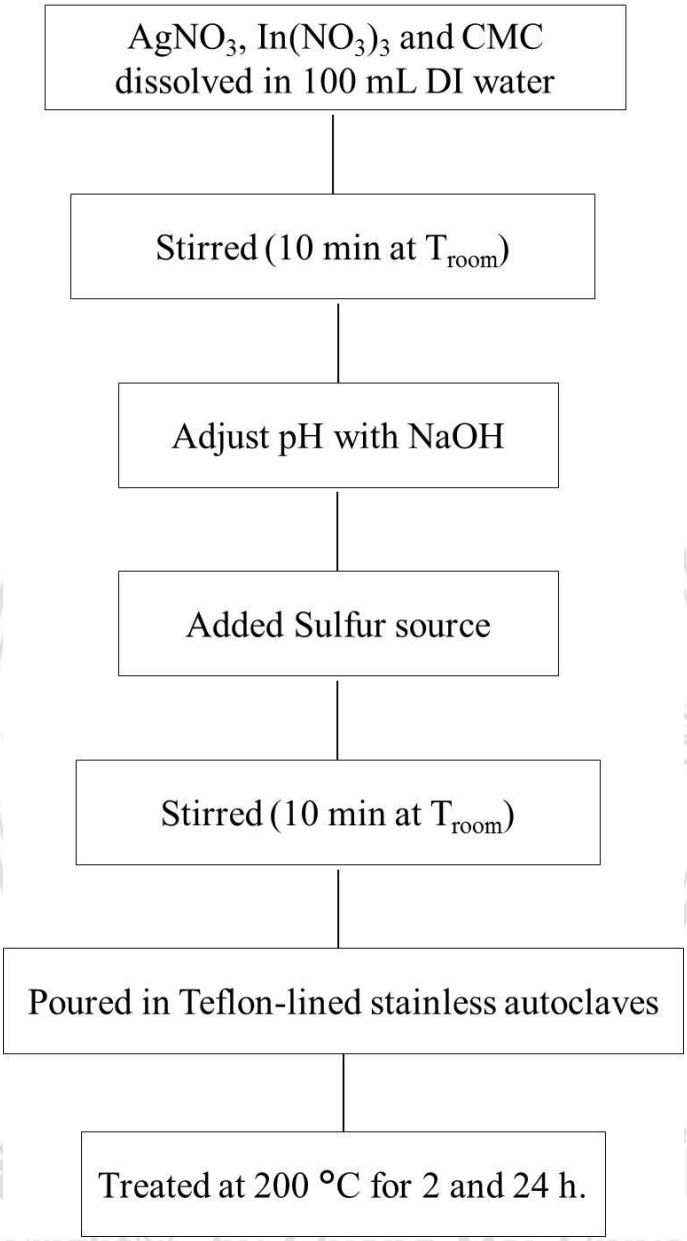

Figure 2.1 Schematic diagram for the synthesis of the CMC-$AgInS_2$ QDs by hydrothermal method.

2.3 Characterization of CMC-$AgInS_2$ QDs

The morphology and nanostructure of the CMC-$AgInS_2$ QDs were observed by using a transmission electron microscope (TEM) operated at 200 kV. The phase and purity of CMC-$AgInS_2$ QDs were studied by using an X-diffractometer using the Ni-filtered monochrome CuK_α radiation. The detection range was 10 – 80 ° with a scanning rate of

1°/s. The phase was identified via the Joint Committee on Powder Diffraction Standards (JCPDS) file no. 25-1328 [49]. X-ray photoelectron spectroscopy (XPS) was conducted using an AXIS Ultra DLD X-ray photoelectron spectrometer. The hydrodynamic size characteristics by using dynamic light scattering (DLS). All measurements were carried out at 25 °C. The functional groups of the CMC-$AgInS_2$ QDs was studied by using a Fourier transform infrared spectrometer with KBr as a diluting agent, and operated in the range of 400 to 4000 cm^{-1}. Thermal analysis was performed using a thermogravimetric analyzer. The TGA curves of the CMC-$AgInS_2$ QDs obtained from heating the samples from 50 °C to 1100 °C at a heating rate of 10 °C/min. The absorption spectra of CMC-$AgInS_2$ QDs were collected using an Agilent 8453 UV-Vis spectrophotometer over the wavelength range from 200 to 800 nm. The emission spectra were collected using a photoluminescence spectrometer at room temperature.

2.4 Drug Encapsulation Studies

0.5 mg of CMC-AIS QDs (sample 8) was dispersed in 200 μL PBS solution (pH 7) and mixed with 800 μL of DOX overnight. After that, the DOX-loaded CMC-$AgInS_2$ QDs were centrifuged and washed with a PBS buffer. The concentration of doxorubicin in the solutions was determined by measuring UV-visible spectrometer absorbance at 480 nm (ε_{480}= 11,500 L/mol cm). The amount of DOX loaded in the CMC-$AgInS_2$ QDs was determined by comparing the amount in the original and the amount in the supernatant and washing solutions. The DOX entrapping efficiency (%DEE) and the DOX loading efficiency (%DLE) were calculated by using the following equations.

DOX entrapping efficiency (%DEE)

$$\%DEE = \frac{\text{Weight of DOX in CMC - } AgInS_2 \text{ QDs} \times 100}{\text{Weight of loaded DOX}}$$

DOX loading efficiency (%DLE)

$$\%DLE = \frac{\text{Weight of DOX in CMC - } AgInS_2 \text{ QDs} \times 100}{\text{Weight of CMC - } AgInS_2 \text{ QDs}}$$

2.5 Cellular Uptake of CMC-$AgInS_2$ QDs

Cellular uptake of CMC-$AgInS_2$ QDs, DOX-loaded CMC-$AgInS_2$ QDs and free-DOX, was studied by using fluorescence microscope. MCF7 cells were cultured in RPMI 1640 containing 10% FBS, 100 U/mL of penicillin, and 0.1 mg/mL streptomycin at 37 °C under 5% CO_2 atmosphere. After 24 h of incubation, the cells were incubated with CMC-$AgInS_2$ QDs, DOX-loaded CMC-$AgInS_2$ QDs and free-DOX, for 5 h (The final concentrations of the free-DOX or DOX-equivalent in the CMC-$AgInS_2$ were 0.60 mM). Finally, the cellular uptake of CMC-$AgInS_2$ QDs, DOX-loaded CMC-$AgInS_2$ QDs and free DOX, were imaged on a fluorescence microscope.

In addition, the cellular drug uptake of DOX-loaded CMC-$AgInS_2$ QDs and free DOX, were also analyzed by using flow cytometer. The cells were seeded in 24 well plates and cultured in the RPMI 1640 medium overnight. The cells were then treated with DOX-loaded CMC-$AgInS_2$ QDs and free DOX for 5 h ([DOX] = 0.60 mM). After washing with the PBS buffer, the cells were resuspended in the PBS buffer, and then analyzed by using the flow cytometer.

2.6 Biocompatibility Studies

MCF7 cells were maintained in the RPMI 1640 medium and cultured in a 24-well plate for 24 h at 37 °C under 5% CO_2 atmosphere for 24 h. Then, various concentrations of CMC-$AgInS_2$ QDs were added to the designated wells at a density of 5×10^4 cells per well. After 48 h of incubation, the culture medium was discarded and the cells were washed twice with PBS buffer and further incubated with 200 mL of solution containing 5 mg MTT/mL phosphate-buffered saline at 37 °C for 4 h. The MTT solution was removed and the cellular formazan cystals were dissolved with 500 mL DMSO for 15 min at 37 °C. The absorbance of formazan solution was measured through UV-Visible spectrophotoscopy at 570 nm with DMSO as blank. Afterward, the biocompatibility was expressed as the percentage of the cell viability.

2.7 Cytotoxicity Studies

MCF7 cells were maintained in the RPMI 1640 medium and cultured in a 24-well plate for 24 h at 37 °C under 5% CO_2 atmosphere for 24 h. Then, various concentrations of DOX-loaded CMC-$AgInS_2$ QDs and free DOX were added to the designated wells at a density of 5×10^4 cells per well. After 48 h of incubation, the culture medium was discarded and the cells were washed twice with PBS buffer and further incubated with 200 mL of solution containing 5 mg MTT/mL phosphate-buffered saline at 37 °C for 4 h. The MTT solution was removed and the intracellular formazan cystals were dissolved with 500 mL DMSO for 15 min at 37 °C. The absorbance of formazan solution was measured through UV-Visible spectrophotoscopy at 570 nm with DMSO as blank. Afterward, the cytotoxicity was expressed as the percentage of the cell viability compared to the control.