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Synthesis and determination of the number of [CdSe] units per CdSe nanoparticle

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ABSTRACT

A method for determining nanoparticle concentration was employed, working on the idea that once the extinction coefficient of the nanoparticles has been determined, the concentration can be obtained by simply taking an absorption spectrum of the sample. The extinction coefficient per mole of CdSe nanoparticle at the first excitonic absorption peak, ϵ was found to be strongly dependent on the size of the nanoparticle, between a square and cubic dependence. The measurements were carried out using purified nanoparticles. A protocol was developed using electroanalysis (ECA) and atomic absorption spectroscopy (AAS) techniques to analyse the Cd^{2+} ion concentration of the CdSe nanoparticles. The prepared CdSe nanoparticles were digested in acid and Cd^{2+} ion present in the solution was determined, it was found practically that there are 1000[CdSe] units per nanoparticle. Theoretical calculations gave 1282[CdSe] units per nanoparticle.

Keywords: Cadmium selenide, nanoparticle, absorption spectrum, atomic absorption spectroscopy (AAS), electroanalysis (ECA).

INTRODUCTION

Determination of the concentration of nanoparticle has proved difficult. The gravimetric methods used to measure the concentration of conventional organic and inorganic compounds have proved problematic. This is because the surface of the nanoparticles contains organic ligands whose identity and quantity are difficult to ascertain at any one time since they readily fluctuate under different conditions (Murray et al., 2001).

A method for determining nanoparticle concentration however has been developed by Yu et al. (2003) working on the idea that once the extinction coefficient of nanoparticles has been determined, the concentration can be obtained by simply taking an absorption spectrum of the sample. They showed that the extinction coefficient of near mono-dispersed CdSe particles increases with an approximate square to a cubic function in the strong quantum confinement regime. They determined the nanoparticle sizes by a combination of transmission electron

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microscopy and X-ray diffraction measurements. Equation 1 is the fitting function of the curve which was obtained. It relates the size D (nm) of the dots in a given sample to the wavelength λ (nm) of the sample's first exciton peak and is valid for samples within the size range of 2 to 6 nm. It is represented as:

$$D = \frac{1.6122}{10^9}\lambda^4 - \frac{2.6575}{10^6}\lambda^3 + \frac{1.6242}{10^3}\lambda^2 - 0.4277\lambda + 41.57 \quad (1)$$

The extinction coefficient per mole of nanoparticles, ε can be calculated using the Beer-Lambert law, shown in Equation 2.

$$A = \varepsilon CL \qquad (2)$$

Where A is the absorbance at the maximum of the first exciton absorption peak for a given sample, C is the molar concentration (mol dm^{-3}) of the same nanoparticle sample and L is the path length (cm) of the illuminating beam as it passes through the sample to record the absorption spectrum. To calculate the extinction coefficient, concentrations of each sample needed to be determined. This was achieved by determining the number of cadmium atoms in a given sample and the size of the nanoparticle. The entire unreacted cadmium precursor had to be removed prior to this analysis by the precipitation and re-suspension technique. As with sizing, the extinction coefficient was determined for a range of samples between the sizes of 2 and 6 nm. The data was again fitted and the relationship between the extinction coefficient and the nanoparticle size was determined for CdSe samples. The relationship is displayed in Equation 3.

$$\varepsilon = 5857\,(D)^{2.65} \qquad (3)$$

Equations 1 and 3 are obtained from the empirical works of Yu et al. (2003). By combining Equations 1-3 above, the concentration of a sample can now be determined by simply taking its absorption spectrum to ascertain the wavelength and the absorbance at the maximum of the first exciton absorption peak. Yu et al. (2003) also showed that the extinction coefficient of CdSe nanoparticles was independent of the surface passivating groups and the solvent in use. It is therefore possible to use the above method to calculate the concentration of CdSe nanoparticle, where the CdSe surfaces may be passivated by different organic molecules or suspended in other solvents.

Determination of the extinction coefficient of semiconductor nanoparticles is of importance for convenient and accurate measurements of the concentrations of nanoparticles. The most important feature of particles in the nanometre regime is that the size determines the particle's electronic properties (Frenzel et al., 2011). Semiconductor nanoparticles have been explored for various applications such as light emitting devices, (Schlamp et al., 1997; Mattoussi et al., 1998; Tessler et al., 2002 and Anantha et al., 2011**)**, lasers, (Klimov et al., 2000 and Kim et al., 2006) and solar cells (Greenham et al., 1996; Chen et al., 2009). Current research on CdSe has focused mostly on the CdSe nanoparticles; the small portions cut out from the bulk CdSe, with diameters between 1 and 100 nm (Boatman et al., 2005). Hence, it is important to know the number of CdSe units per nanoparticle because, the interest in these nanosized systems can be understood by their special properties, which are significantly different from those of the parent bulk compound; and they are open to the possibility of novel technological applications (Kandasamy et al., 2009). For example, cadmium selenide nanoparticles exhibit novel optoelectronic properties for potential biomedical applications (Bruchez et al., 1998 and Bacherikov et al., 2006). However, their metabolic stability is not fully understood because of the difficulties in measuring the free Cd^{2+} ions from biological tissues of exposed individuals (Arslan et al., 2011).

In this work we will employ the hot injection technique (Wang and Herron, 1991 and Alivisatos, 1996) to synthesize high quality CdSe nanoparticles. Their extinction coefficient will be determined in the size range of 2 to 6 nm and an analytical protocol will be developed to determine the composition of the CdSe units per nanoparticle. The composition will also be determined theoretically. These procedures will give the number of CdSe units per CdSe nanoparticle, providing information which could be used for quantum size and safety studies of the CdSe nanoparticles. We will also determine the nature and size of the purified nanoparticles, and the influence of increasing the amount of CdSe in the nanoparticle on the absorbance.

MATERIALS AND METHODS

Equipment

Atomic Absorption Spectrophotometer (Unicam 919, Cambridge), UV–Visible Spectrometer (Perkin Elmer Lambda Bio 10), High Resolution-Transmission Electron Microscope (JOEL JEM 2010), X-Ray analysis system (Oxford Instrument ISIS 310), Potentiostat (EG&G Princeton Applied Potentiostat/Galvanostat model 210), Electrodes (Saturated Calomel/Platinum Mesh Electrodes).

Chemicals

Trioctylphosphine oxide (TOPO, 90%), trioctylphosphine (TOP, 90%), hexadecylamine (HDA, 90%), and selenium powder (99.999%) were purchased from Aldrich. Cadmium acetate (anhydrous, 99.99+%, ChemPur), n-tetradecylphosphonic acid (TDPA, 98%, AlfaAesar), toluene (HPLC, Rathborne), methanol (HPLC, Rathborne) were also purchased. All the chemicals were used as received from the suppliers.

Synthesis of TOPO stabilised CdSe nanoparticles

The reaction took place in a three-neck round-bottom flask (Figure 1). One arm of the flask was fitted with a reflux condenser, one with a rubber septum through which reagents could be injected or products removed, and the third with a temperature probe. The flask, which was maintained under an inert atmosphere, was heated by a heating mantle. All the chemicals were dried and degassed before use in order to achieve water and oxygen free conditions. The cadmium and selenium containing precursors, TOPCd and TOPSe respectively, were prepared under argon in a dry box in which the water content was controlled to less than 20 ppm. 8 g of TOPO were dried and degassed under vacuum at 180°C for about 1 hour. The TOPO was then cooled to between 80 and 120 ºC; 5 g of HDA and 0.15 g of TDPA were added and the drying process was continued at 120 ºC under vacuum for 20 minutes.

Trioctyl phosphine selenide (TOPSe) solution which had been prepared by dissolving 0.158 g of selenium in 2 ml TOP was added to the mixture. The temperature of the mixture was raised to 300 ºC and the reaction mixture was allowed to attain this temperature. The thermostat of the heating mantle was reduced to 260 ^{o}C and trioctyl phosphine cadmium (TOPCd) solution which had been prepared by adding 0.120 g of cadmium acetate to 3 ml of TOP was rapidly injected into the mixture with vigorous stirring. This resulted in immediate nucleation of the CdSe nanoparticles and a fall in temperature. The nanoparticles were allowed to grow for 30 minutes before stopping the process by quenching with cold toluene. The nanoparticles produced were stabilised by TOPO, TOP, HDA and TDPA all of which have functional groups capable of binding to the CdSe surface; and are called TOPO stabilised CdSe nanoparticles.

Purification of CdSe nanoparticle

The TOPO stabilised CdSe nanoparticles were purified by precipitation with methanol and allowed to settle overnight by gravity. The process of separation with methanol and re-suspension in toluene was repeated three times to ensure complete removal of any unreacted precursor. The pure nanoparticles were dried on a hot plate at 60 ^{o}C in a fume cupboard.

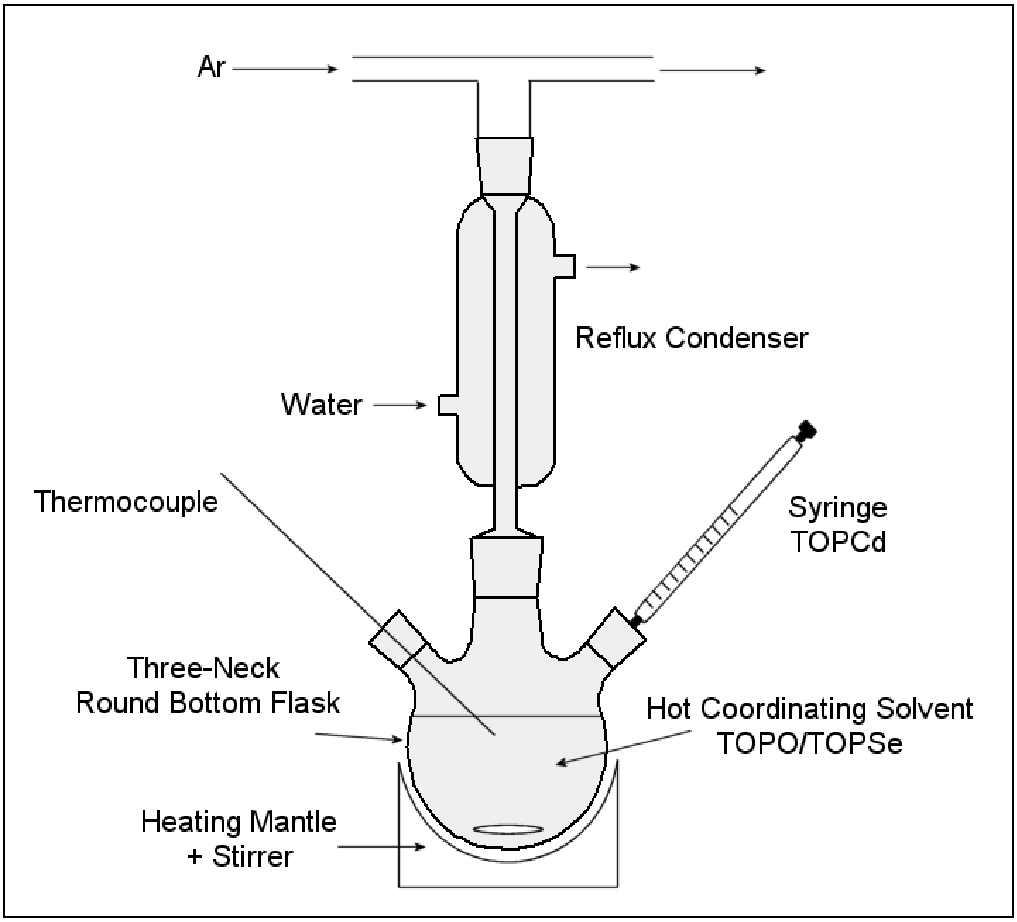

Figure 1: Schematic of apparatus for preparing CdSe Nanoparticles.

Digestion

High purity concentrated nitric acid (2 ml) was added to a designed safety tube containing the dry quantum dots and the solution was allowed to sit overnight. 6 drops of high purity concentrated hydrochloric acid were added to the tube and it was placed on a hot plate set at 80 °C for about 2 hours until the solution became clear and contained no more solids. The tube was designed in such a way that the toxic H_2Se vapour released was forced by argon gas into a concentrated mixture of NaOH and $ZnCl_2$ solutions. This produced ZnSe that was less toxic and in a form that could be safely disposed of. The digested solution was then diluted and kept for cadmium ion analysis.

Analytical protocol to determine the composition of CdSe nanoparticles

Cadmium selenide nanoparticles produced in a reaction time of 30 minutes were analysed as described bellow:

UV-Vis absorption spectra were determined for nanoparticles ranging from 0.25 mg to 1.25 mg. The crystalline state of the purified nanoparticles was determined using the high resolution-transmission electron microscope (HR-TEM), while their elemental composition was determined using x-ray diffraction (EDX).

The concentrations of cadmium ions in aliquots of the digested solutions of the CdSe nanoparticles were determined using electro-analysis and atomic absorption spectroscopy.

Calibration curves for the two methods were first obtained as follows:

- Anodic stripping voltammograms were recorded at a scan rate of 20 mVs^{-1} and deposition time of 80 s at different applied potentials relative to a saturated calomel reference. Different volumes of standard cadmium nitrate solution of concentration 0.05 mol dm^{-3} were added to the cell to give a range of concentrations of cadmium ions. The voltammograms obtained were analysed to give the peak area (charge) associated with stripping the cadmium deposited. The charge required to strip the cadmium was then plotted against Cd^{2+} ion concentration to obtain a calibration curve.
- For the AAS method, the variation in the concentration of the Cd^{2+} ions was obtained by using varying volumes of 1000 ppm standard solution of cadmium ions.

Both calibration curves are presented in the appendix.

The curves were then used to determine the concentration of cadmium ions present in the aliquots of the digested solutions.

RESULTS

Analyses of nanoparticles

Figures 2 to 4 present the analyses of cadmium selenide nanoparticles produced in a reaction time of 30 minutes.

The UV-Visible absorption spectra of cadmium selenide nanoparticles are shown in Figure 2. The result showed that, for any given wavelength, the absorption increased with an increase in the concentration of the CdSe in the nanoparticles. This trend confirms the Beer-Lambert law given in Equation 2.

The transmission electron microscopy, HR-TEM and x-ray diffraction, EDX measurements are presented in Figures 3 and 4 respectively. The HR-TEM of the sample showed that the CdSe nanoparticles were crystalline in nature, having well-defined lattice fringes. The particle size as determined by TEM was 5.2 ± 0.3 nm. Using the experimental conditions of wavelength and extinction coefficient, the particle size of the prepared CdSe nanoparticles as calculated from empirical equations of Yu et al. (2003) using Equations 1 and 3 was 5.1(6) ± 0.02 nm and this compares favourably well with the 5.2 ± 0.3 nm from the TEM data. From the energy dispersive x-ray analysis spectra shown in Figure 4, it is seen that the CdSe nanoparticles are made up of the elements cadmium and selenium. The TOPO molecules attached to the nanoparticles are not indicated because; TEM does not resolve them (Striolo et al., 2002).

From the experimental values of the particle size, the extinction coefficient, and hence, the concentration of the CdSe nanoparticle were calculated using the Beer-Lambert law (Equation 2). Figure 5 gives a relationship between the concentration of CdSe nanoparticles and their absorbance. The result further confirmed the Beer-Lambert law by showing that as the concentration of CdSe nanoparticles increased it absorbance also increased.

Standard cadmium calibration curves using the electro-analyses and AAS methods gave straight line graphs, whose equations can be given respectively as

$$Charge = 19995\left[Cd^{2+}\right] \quad (3)$$

And $Absorbance = 0.0891\left[Cd^{2+}\right]$ (4)

They were used to determine the cadmium ion concentration by simply using the Equation 5 for the electro-analyses method and Equation 6 for the AAS analysis given below.

$$\left[Cd^{2+}\right] = \frac{Charge}{19995} \quad (5)$$

$$\left[Cd^{2+}\right] = \frac{Absorbance}{0.0891} \quad (6)$$

Where Charge = Charge or area below each voltammogram, and

$[Cd^{2+}]$ = Cadmium ion concentration that caused the voltammogram.The cadmium ion concentrations were those of Cd^{2+} present in the cell used for the analysis. To get the actual concentration of cadmium in the samples, the concentration of Cd^{2+} in the cell was multiplied by a dilution factor, DF calculated as in Equation 7 (Ndifor and Anagho, 2012).

$$DF = \frac{V_{NaCl} + V_{HgCl_2} + V_{Cd(NO_3)_2}}{V_{Cd(NO_3)_2}} \qquad (7)$$

Analysis of the data obtained using the above equations gave the plots shown in Figure 6. Relating the concentration of CdSe obtained from Beer-Lambert's law and the cadmium ion concentration from electro analysis and AAS studies gave the plot in Figure 7.

From Figures 7 and 8, the equation relating the cadmium ion and cadmium selenide concentrations is:

$$[CdSe] = 0.001[Cd^{2+}] \qquad (8)$$

From this equation, it can be deduced that the concentration of cadmium ions in a nanoparticle is, $[Cd^{2+}] = 1000[CdSe]$.

This implies that experimentally, one CdSe nanoparticle contains 1000 units of CdSe.

Theoretical calculation of the number of CdSe units per canoparticle

For the purpose of comparison, the above quantity was also deduced theoretically as follows:

$$\text{Volume of a CdSe particle} = \frac{4}{3}\pi r^3$$

$$\text{Mass of one CdSe particle} = \frac{4}{3}\rho\pi r^3$$

$$\text{NCdSeUON} = \frac{4}{3}\rho\pi r^3 \frac{N_A}{M_{CdSe}}$$

Where, NCdSeUON is Number of CdSe units in one nanoparticle, ρ is the density of CdSe in g cm^{-3}, r is the radius in cm while M_{CdSe} is its molar mass in g mol^{-1}.

Density of CdSe = 5.655 g cm^{-3}, molar mass of Cd = 112.4 g mol^{-1}; molar mass of Se = 78.9 g mol^{-1}, M_{CdSe} = 112.4 + 78.9 = 191.3 g mol^{-1}; $N_A = 6.022 \times 10^{23}$ mol^{-1}

The diameter, D, of CdSe was calculated from the empirical relation of Yu et al. (2003) given in Equation 1, using the experimental wavelength (λ) of 612 nm. The value of D obtained is 5.162 nm or 5.162 x 10^{-7} cm.

Therefore, Number of CdSe units in one nanoparticle

$$= \frac{4}{3}\pi \times 5.655 \times \left(\frac{5.162 \times 10^{-7}}{2}\right)^3 \times \frac{6.022 \times 10^{23}}{191.3} = 1282$$

This shows that there are 1282[CdSe] units in one CdSe nanoparticle.

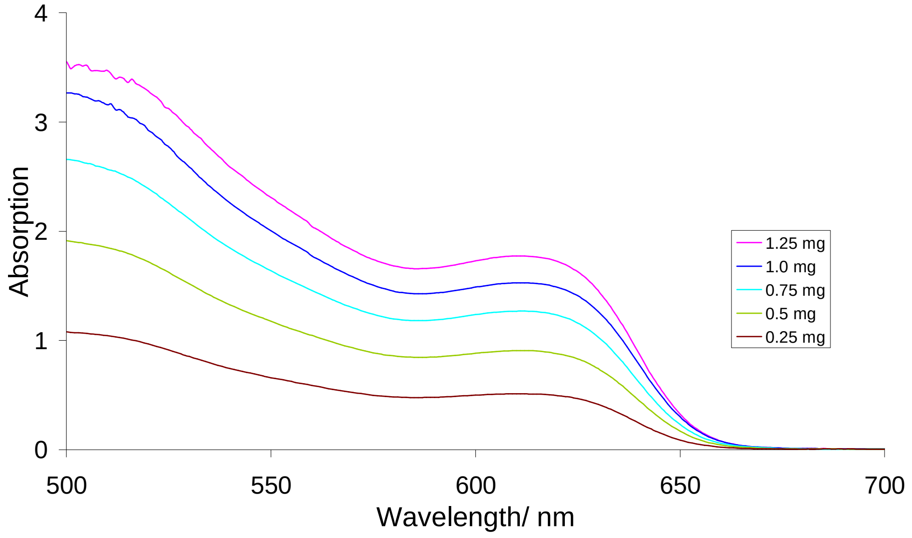

Figure 2: UV-Vis absorption of CdSe nanoparticles sample after 30 minutes growth using different masses of CdSe nanoparticles in 5 cm^3 of solvent.

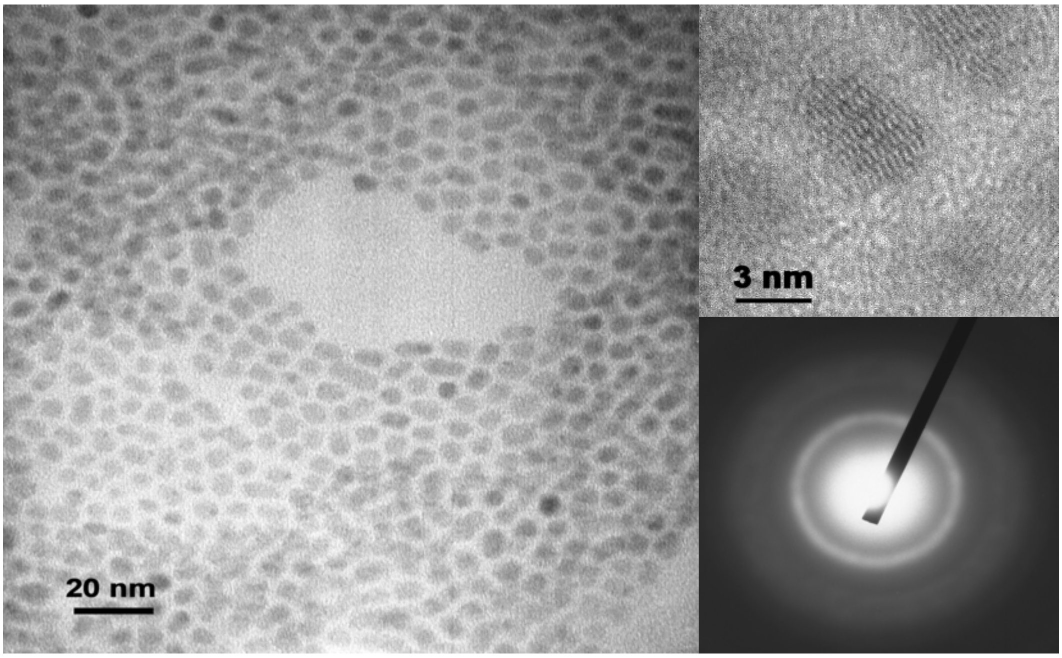

Figure 3: HR-TEM of CdSe nanoparticles after 30 minutes growth showing it crystalline nature.

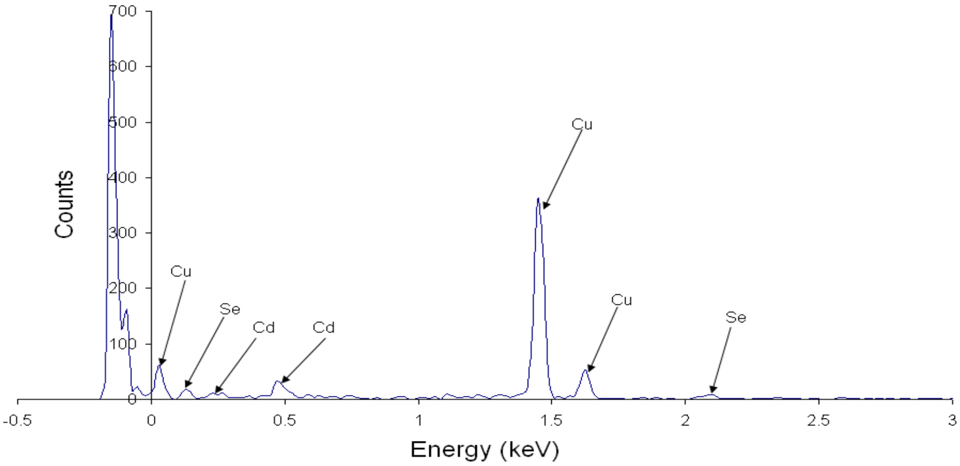

Figure 4: EDX spectra of CdSe nanoparticle confirming the elemental composition of cadmium and selenium present in the nanoparticle.

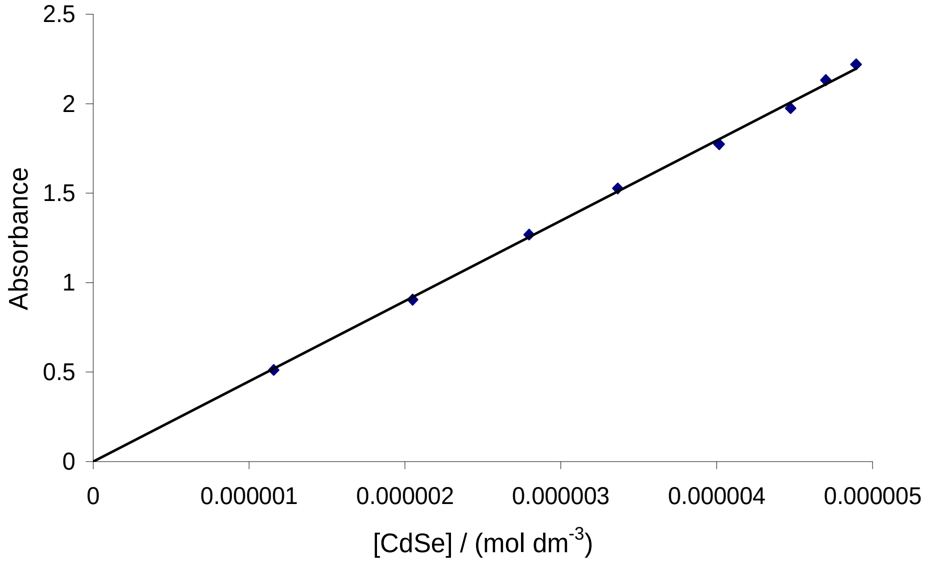

Figure 5: Relationship between concentrations of CdSe nanoparticle and their absorbance. It shows that Beer-Lambert's law is valid over the concentration range employed.

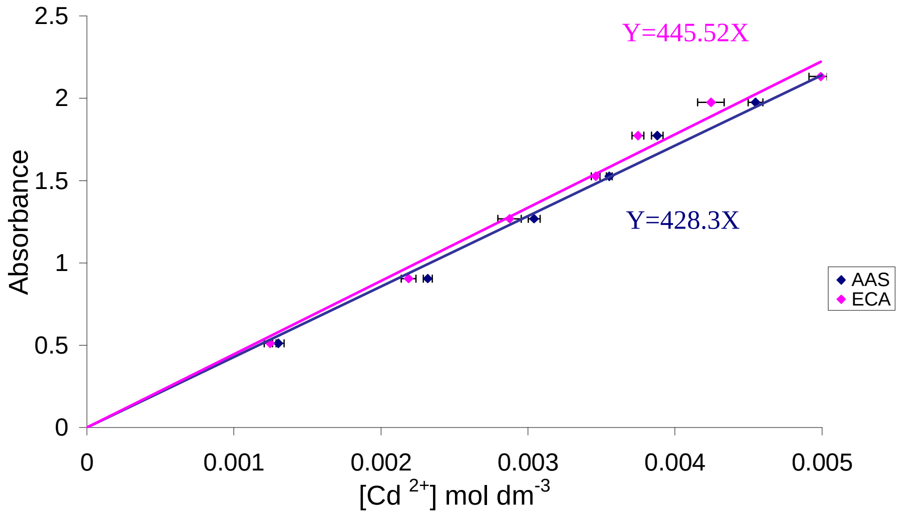

Figure 6: Concentration of cadmium ion using the electro analytical and AAS methods. The curve gives a linear relationship in which the concentration of cadmium ion is related to it absorbance.

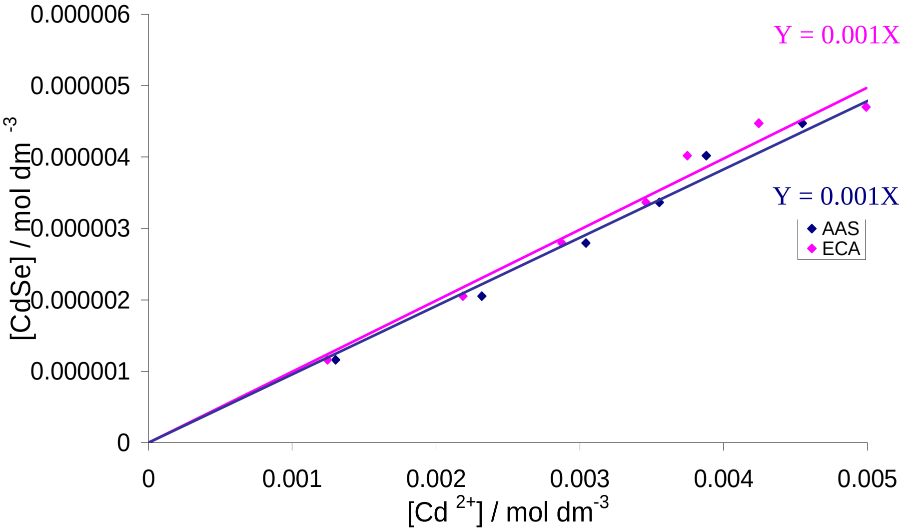

Figure 7: Relationship between CdSe particle concentration obtained from the absorbance equation and cadmium ion concentration obtained from the electro analytical and AAS methods.

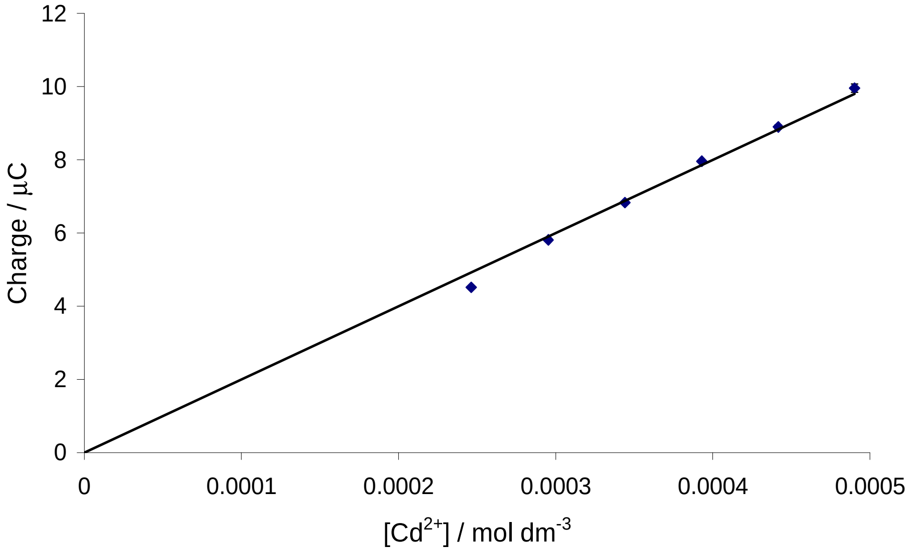

Figure 8a: Standard cadmium calibration curve by the electro analytical method using different cadmium ion concentration and fixed mercury ion concentration. The curve gives a linear relationship in which cadmium ion concentration in the analysis cell can be calculated by dividing the charge obtained by 19995.

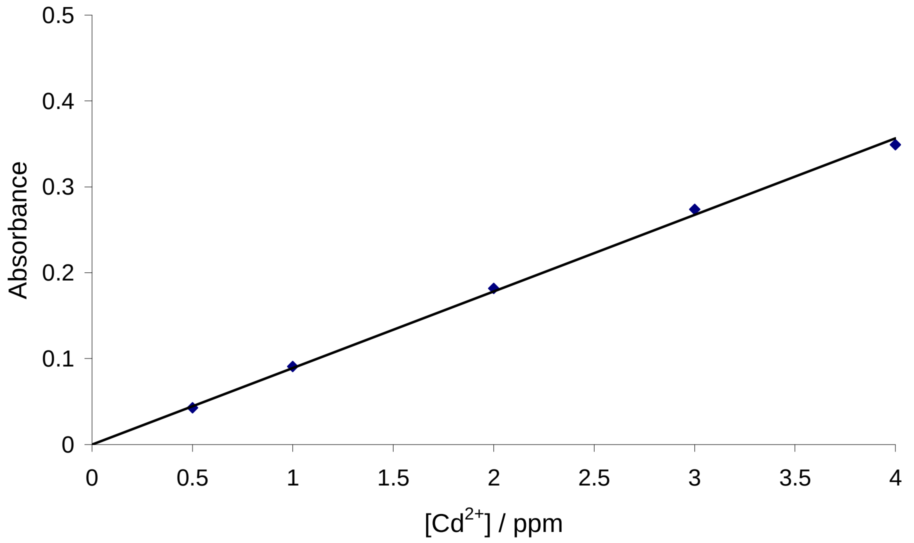

Figure 8b: Standard cadmium calibration curve by the AAS method using known cadmium concentrations. The curve gives a linear relationship in which the concentration of cadmium in the analysis solution can be calculated by dividing the absorbance obtained by 0.0891.

DISCUSSION

Synthesis of TOPO stabilised CdSe nanoparticles

The hot injection method for preparing CdSe nanoparticles was first reported by Murray and co-workers (1993).

The schematic representation of the synthesis of the TOPO stabilised nanoparticles can be written as in Figure 9.

The reagents, TOPCd and TOPSe were initially prepared from the precursor organic liquids, trioctylphosphine oxide (TOPO) and trioctylphosphine (TOP). During the growing process, it was observed that the size of the CdSe nanoparticles produced increased with increasing growing time. However, further analysis of the nanoparticles, used particles grown for a period of 30 minutes.

The synthesis took place in the presence of a number of organic liquids. These organics played major roles in the synthesis. The two most important compounds, TOPO and TOP acted in various ways during and after the nanoparticle growth to ensure quality and stability (Murray et al., 1993).

- They acted as a high temperature solvent in which growth of the nanoparticles occurred;
- they mediated the growth of the nanoparticles by complexing with the cadmium and selenium ions, thereby controlling the rate at which they were released to bind to the nanoparticle surface;
- they bound to the CdSe sterically, stabilising them against irreversible aggregation;
- they passivated the surfaces to relieve the high surface energy generated due to the extremely small size of the nanoparticles.

Hexadecylamine (HDA), a long chain amine and tetradecylphosphonic acid (TDPA), a long chain phosphonic acid, were the other organic molecules used together with TOPO and TOP (Ruach-Nir et al., 2003). HDA improved CdSe growth dynamics, preventing defocusing of the nanoparticle size distribution, and eliminating the need for any post preparation size selective precipitation to improve mono-dispersivity, while TDPA slowed down the rate of nanoparticle growth, resulting in the prepared nanoparticles having better crystallinity.

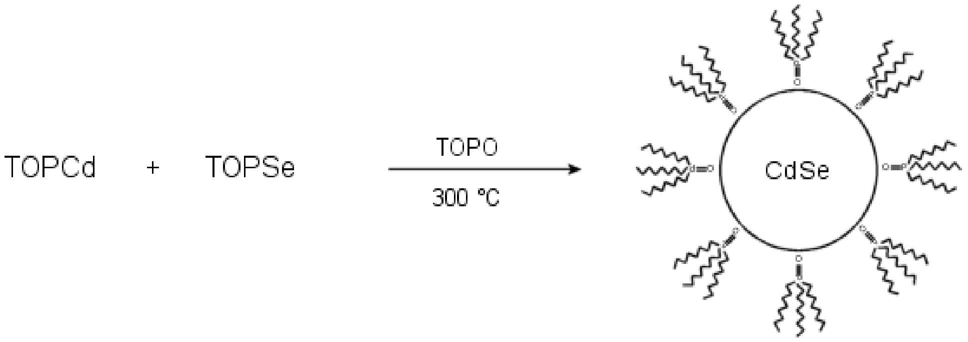

Figure 9: Schematic representation of the preparation of CdSe nanoparticles by the hot injection techniques.

All the organic molecules used in the hot injection preparation are surfactants composed of a polar head group and one or more hydrocarbon organic chains forming the hydrophobic end of the molecules. During the synthesis, these molecules are continuously adsorbing and desorbing from the nanoparticle surface allowing them to grow in a controlled manner.

Analyses of CdSe nanoparticles

The crystalline nature of CdSe nanoparticles confirms the earlier observation by Murray et al. (2003). The particle size as determined by TEM of 5.2 ± 0.3 nm or the value of 5.1(6) ± 0.02 nm obtained using the experimental conditions of wavelength and extinction empirical equations of Yu et al. (2003) given by Equations 1 and 3 are about 20 percent larger than the 4.1 ± 0.5 nm of Okuda et al. (2010) determined using the biotemplated synthetic technique. This suggests that the CdSe nanoparticle size depends on the method of synthesis.

The cadmium ion concentration using both the electrochemical and atomic absorption spectroscopy methods of analysis had a linear relationship with cadmium selenide Q-dot concentration with a gradient of 0.001. This implies that there are 1000[CdSe] units per nanoparticle. Theoretically the [CdSe] units per nanoparticle calculated by taking the volume and density of the CdSe particle and the Avogadro number into consideration gave 1282 [CdSe] units per nanoparticle. This value is just over 28 percent greater than the results obtained experimentally. This difference is justified since a lot of approximations are made in the theoretical calculations.

The need to determine CdSe nanoparticle size is important because such knowledge facilitates an assessment of its doping capacity. For example, Acharya et al. (2011) prepared CdSe and polythiophene nanocomposites and found that reduction of band energy is observed on addition of polymer polythiophene with CdSe nanoparticle. Cd^{2+} is toxic, so knowing the number of [CdSe] units per nanoparticle will be helpful in toxicological studies. Wang et al. (2008) showed that the cytotoxicity of CdSe nanoparticles is modulated by surface coatings.

Conclusion

CdSe nanoparticles have been prepared by the hot injection technique. In this paper we produced monodispersed crystalline CdSe nanoparticles with a size of 5.2 ± 0.3 nm as determined by TEM and 5.1(6) ± 0.02 nm as calculated from the maximum absorption wavelength of 612 nm obtained from UV- Vis studies. Elemental analysis of the nanoparticles practically using mercury film electrochemical and the atomic absorption spectroscopy techniques gave 1000[CdSe] units per CdSe nanoparticle. We believe that this study will be helpful in tuning the size of CdSe nanoparticles for different technological applications and also provide useful safety information for potential biomedical application since cadmium is a toxic heavy metal and selenides are toxic in large amounts.

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