Biomolecularly capped uniformly sized nanocrystalline materials: glutathione-capped ZnS nanocrystals

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Abstract. Micro-organisms such as bacteria and yeasts form CdS to detoxify toxic cadmium ions. Frequently, CdS particles formed in yeasts and bacteria were found to be associated with specific biomolecules. It was later determined that these biomolecules were present at the surface of CdS. This coating caused a restriction in the growth of CdS particles and resulted in the formation of nanometre-sized semiconductors (NCs) that exhibited typical quantum confinement properties. Glutathione and related phytochelatin peptides were shown to be the biomolecules that capped CdS nanocrystallites synthesized by yeasts Candida glabrata and Schizosaccharomyces pombe. Although early studies showed the existence of specific biochemical pathways for the synthesis of biomolecularly capped CdS NCs, these NCs could be formed in vitro under appropriate conditions. We have recently shown that cysteine and cysteine-containing peptides such as glutathione and phytochelatins can be used in vitro to dictate the formation of discrete sizes of CdS and ZnS nanocrystals. We have evolved protocols for the synthesis ZnS or CdS nanocrystals within a narrow size distribution range. These procedures involve three steps: (1) formation of metallo-complexes of cysteine or cysteine-containing peptides, (2) introduction of stoichiometric amounts of inorganic sulfide into the metallo-complexes to initiate the formation of nanocrystallites and finally (3) size-selective precipitation of NCs with ethanol in the presence of Na+. The resulting NCs were characterized by optical spectroscopy, high-resolution transmission electron microscopy (HRTEM), X-ray diffraction and electron diffraction. HRTEM showed that the diameter of the ZnS–glutathione nanocrystals was 3.45 ± 0.5 nm. X-ray diffraction and electron diffraction analyses indicated ZnS–glutathione to be hexagonal. Photocatalytic studies suggest that glutathione-capped ZnS nanocrystals prepared by our procedure are highly efficient in degrading a test model compound.

1. Introduction

Nanocrystalline materials (NCs) have become one of the most intensely studied classes of materials in the past 20–30 years [1–20]. Much of the interest in NCs has been inspired by their potentially unique electronic and optoelectronic applications. Recent investigations have suggested the applicability of NCs in the conversion of solar radiation [9, 21–23], reduction of metal ions [9, 24, 25], nucleic acid hybridization studies [26–30], drug formulation applications [31–36], remediation of hazardous environmental contaminants such as pesticides and purification of water [37–41]. A variety of other medical applications for NCs is conceivable. For example, treatment of specific dermatological conditions might be achievable using ZnS NCs. Psoriatic skin has been treated with UV in the presence or absence of DNA damaging agents [42]. Such treatments could potentially benefit from the inclusion of ZnS NCs. These materials will produce free radicals that can destroy the psoriatic cells upon UV irradiation in situ. Further damage to the normal skin could then be prevented by the application of free-radical scavengers such as glutathione (GSH) [43]. As explained later, GSH has proven to be a very important ingredient in the engineering of size-controlled production of NCs.

Size dependence of optical, electronic and possibly other properties of NCs dictates that the sizes of the manufactured materials exhibit a narrow distribution [1–6, 8–12, 44–47]. Large variations in the sizes of NCs within a given batch may render them of little use in many applications. Consequently, a variety of physicochemical, electrochemical and mechanical procedures have been devised for the production of NCs within a narrow size distribution range [46–53]. The syntheses of NCs have
been accomplished in both aqueous and non-aqueous media [44, 51, 54]. Two important aspects of NC syntheses relate to (i) preventing the growth of particle size and (ii) isolation of particles in a narrow size-distribution [1–6, 11, 12, 44– 50]. Successful approaches for the syntheses of NCs have involved ‘capping’ the particles with suitable organic species to prevent particle growth. In addition, isolation of NCs within a narrow size distribution range has been accomplished by precipitation procedures [2, 5, 9, 44, 45]. Capping of NCs has been more easily accomplished in non-aqueous media using organic species as capping agents [9, 44, 45].

Novel strategies for NC syntheses emerged from apparently unrelated biological studies on in vivo detoxification of cadmium ions in certain yeasts [55–60]. The precipitation of metal sulfides in yeasts has been known for a long time. However, it was shown only recently that in many cases such metal sulfides, particularly CdS, were nanocrystalline in nature [60, 63]. Studies on yeasts C. glabrata and S. pombe established that the formation of CdS NCs proceeds in several steps that have been evolved by these organisms to counter toxic effects of Cd. One of the main mechanisms employed by living organisms to counter the toxic effects of free cadmium ions involves complexation of the metal ion by peptides or proteins [61–64]. The tripeptide GSH chelates and detoxifies Cd (II) in most studied organisms [43]. In addition, GSH-related peptides known as phytochelatins (PCs) chelate Cd (II) in plants and selected yeasts [61–68].

Some of the earliest studies documented the presence of acid labile inorganic sulfide in Cd–PC complexes [56–60, 69]. Later studies showed that Cd–PC complexes isolated from yeasts exhibited optical and other properties akin to those of nanocrystalline CdS [55, 59, 60, 63–69]. More recently, formation of nanocrystalline CdS has also been documented in bacteria [70–72]. However, the composition of such bacterial CdS particles in terms of their capping materials has not been established so far. There is considerable evidence to show that in vivo synthesis of CdS NCs proceeds in two steps. Cd–PC or Cd–GSH complexes are formed in the first step, with subsequent introduction of sulfide being the second step [63–68]. Additional studies showed that the yeasts may also affect a change in capping material depending on the growth conditions and the age of the yeast culture [63]. For example, CdS particles formed in early log phase cultures of C. glabrata contain GSH as capping material. PCs replace GSH as the capping material in older cultures [63].

Studies on Cd-sensitive mutants of S. pombe established specific biochemical pathways that were required for the synthesis of CdS NCs [61]. However, these biochemical pathways do not appear to be obligatory for the formation of NCs in vitro. Preliminary in vitro studies established that inorganic sulfide could be introduced into preformed Cd–GSH or Cd–PC complexes under defined reaction conditions to form CdS NCs [55, 60]. Recent studies from our laboratory have defined many of the reaction conditions that are required for the reproducible synthesis of cysteine, GSH or PC-capped NCs [73–77]. We have established a three-step process for the synthesis of NCs in a narrow size range [75–77]. In this paper, we discuss the use of the tripeptide GSH to cap ZnS NCs. A size-selective isolation procedure is then used to further limit the size distribution of the particles. Furthermore, the ability of these NCs to act as photocatalysts is demonstrated by the photodegradation of the model organic compound p-nitrophenol (PNP).

2. Materials and methods

2.1. Materials

Analytical grade chemical reagents were procured from Fisher Scientific. GSH and Sephadex G-25 (fine) were purchased from Alexis and Sigma, respectively. Copper grids for electron microscopy were obtained from Electron Microscopy Sciences (Fort Washington, USA).

2.2. Synthesis of ZnS–GSH nanocrystallites

Zn\(_2\)(GSH)\(_2\) complex was prepared by titrating stoichiometric amounts of Zn (II) as ZnSO\(_4\) (freshly prepared in 0.01N HCl) into a degassed solution of GSH in 1M Tris.Cl, pH 8.6. It is critical that the GSH solution is prepared in a degassed buffer and used immediately to prevent relatively
rapid oxidation of GSH under alkaline conditions. Inorganic sulfide as sodium sulfide was then titrated into the metallo-peptide complex by stirring-in suitable aliquots of sodium sulfide to obtain Zn:sulfide molar ratios varying from 1:0.25 to 1:5.0. All titrations were performed with vigorous mixing. The tubes were then capped and incubated at room temperature for 30 min and then at 37 °C for further 30 min. After incubation, the reaction tubes were bubbled with nitrogen for 10–15 min to remove unreacted sulfide. Thus formed ZnS nanocrystals were selectively precipitated by ethanol following the addition of 3M sodium acetate, pH 6 (110 µl ml⁻¹). Ethanol was added drop-wise to the sample until it turned slightly cloudy. The resulting sample was centrifuged in a Beckman Model TJ-6 at 3000 rpm for 20 min. The pellet was dissolved in 1 ml of degassed 10 mM Tris.Cl, pH 8.6 and re-precipitated as described above. This process of ethanol precipitation and resuspension was repeated four times. In some cases, formation of nanocrystals was allowed to proceed at 35 °C for one hour.

Gram-scale quantities of NCs were prepared by scaling up the above-described procedures. The NCs precipitated with ethanol were washed with 70% ethanol several times and then dried using a Virtis freeze-drier. Powdered NCs or solutions of NCs were stored at 4 °C until characterization studies.

2.3. Analysis of size distribution by gel filtration

Compositional and size-distributional analyses on the ethanol-fractionated samples were carried out using gel filtration as described earlier [73–77]. The samples were chromatographed on a Sephadex G-25 column (1.6 × 30 cm) using 10 mM Tris.Cl, pH 8.6 as eluent. The individual fractions (~1.0 ml) were analysed for Zn, sulfide and GSH concentrations. Zinc concentrations were determined on appropriately diluted samples by atomic absorption on a Perkin–Elmer atomic absorption spectrophotometer (Model 3100). Sulfide and GSH assays were performed.

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**Figure 2.** Gel filtration composition profiles of ZnS–GSH nanocrystallites prepared without ethanol treatment. Profiles for samples prepared with initial Zn:sulfide ratios of 1:0.25, 1:0.5, 1:1, 1:1.5 and 1:5 are shown in (a)–(e), respectively.
Figure 3. UV/Vis absorption spectra of ZnS–GSH nanocrystallites on non-precipitated samples. The column fractions obtained after gel filtration as shown in figure 2 were scanned. The spectra for fractions from samples prepared with initial Zn:sulfide ratios of 1:0.25, 1:0.5, 1:1, 1:1.5 and 1:5 are shown in (a)–(e), respectively.

as detailed previously [73–77]. Samples prepared without ethanol precipitation were also analysed for composition and size distribution to demonstrate the significance of ethanol/Na⁺ precipitation in limiting size distribution.

2.4. UV–Vis and fluorescence spectroscopic analyses of nanocrystals

Optical spectroscopic procedures were performed as detailed previously [73–77]. Perkin–Elmer spectrofluorimeter LS50B controlled by FLDM software was used to record luminescence spectra, whereas Lambda 3B was used for UV–Vis spectroscopy. For luminescence spectra, the samples were excited at 275 nm and a 290 nm emission filter was used to block secondary emissions. Samples used for optical spectroscopy generally contained 20 µg ml⁻¹ of Zn as ZnS–GSH in 10 mM Tris.Cl, pH 8.6.

2.5. Electron microscopic and powder x-ray diffraction analyses

A packed powder mount of ZnS–GSH sample was prepared according to the procedures described previously [78]. The mounts were run from 2–72° 2θ with a 0.02° step size and 4 s per step on a Siemens D-500 x-ray diffractometer using CuKα radiation. The resulting data were compared with the standard data contained in a Powder Diffraction File [79].

High-resolution transmission electron microscopy (HRTEM) analyses and selected electron diffraction (SAED) analyses of NCs were performed on a Phillips CM300 transmission electron microscope operating at 300 kV. ZnS–GSH particles were deposited from aqueous solutions onto 300 mesh copper grids coated with an ultrathin carbon support film. Appropriate dilutions of the ZnS–GSH NCs were placed on the grid. The grids were allowed to air-dry at room temperature. Size measurements on NCs were
made by analysing HRTEM lattice images of 100 particles from different fields of the grid. The chosen fields were demonstrated to be consistent in terms of the distribution of the material on the entire grid.

2.6. Photodegradation studies

PNP was used as a model compound to study the ability of various nanocrystals to photodegrade organics. A Glo-Mark Systems Inc. 8 W shortwave ultraviolet lamp was used for initiating the photodegradation process. This lamp has an approximate photon flux of $41 \pm 1 \mu$Einst $m^{-2} s^{-1}$ at a distance of 1 cm. All experiments were set up in quartz cuvettes [73–77]. Various components of the reaction were mixed in a final volume of 2 ml. The final concentrations of PNP and Zn (as GSH–ZnS) in most experiments were 50 and 500 nmoles ml$^{-1}$, respectively. This resulted in a substrate to catalyst ratio of 1:10. This ratio is substantially lower than that used by others [37–41, 80, 81, 93]. Dieckman and Kimberly [80] used 0.5 mM PNP and 32 mM of TiO$_2$ which yields a ratio of 1:63 (substrate:catalyst). Akmehmet and Inel [93] used a catalyst:substrate ratio of 750:1. Vinodgopal et al [81] have used similar concentrations of TiO$_2$ for the photodegradation of other organic materials at catalyst-to-substrate ratios varying from 125:1 to 625:1.

The reaction mixtures were exposed to the UV lamp in quartz cells. The concentrations of PNP were monitored by reading $A_{405}$ at predetermined intervals. Various experiments were performed to determine the influence of parameters such as pH and composition of NCs.
3. Results and discussion

3.1. Synthesis of GSH-capped NCs

Most of the initial studies on the formation of CdS NCs by living yeasts were aimed at understanding the mechanisms of metal ion detoxification [82–84], although photochemical implications of NCs in living cells have also been discussed [70, 71]. Evolution of H₂S upon acidification of Cd-binding peptides from *S. pombe* was the first indication of the presence of CdS in these complexes [56, 85, 86]. Later, combination of gel filtration chromatographic analyses and optical spectroscopy led to the suggestion that Cd–PC complexes contained CdS NCs [58–60]. These suggestions were confirmed by additional characterization studies [63, 74]. As mentioned earlier in this paper, genetic regulation of CdS NC synthesis *in vivo* was suggested by the isolation of mutants unable to produce these particles [82–84]. However, CdS NCs could be formed in vitro by reacting preformed Cd–GSH or Cd–PCs with sulfide [74]. CdS NCs formed *in vivo* or *in vitro* were shown to be heterogeneous in size and composition by chromatographic and chemical analyses [60, 74].

Our studies showed for the first time that the nature of capping material could dictate the size and the extent of size distribution in CdS NCs. For example, a GSH cap resulted in the formation of CdS particles that were significantly heterogeneous in both size distribution and a relatively constant chemical composition [74]. In contrast, CdS NCs formed with PCs showed remarkably narrow size distribution and a relatively constant chemical composition [74]. In addition, we showed that the use of cysteine-containing peptides allowed exchange of capping materials [74].

These studies on the synthesis of CdS NCs by using appropriate capping materials laid the foundation for
our recent studies on the formation of ZnS NCs using biomolecules like cysteine and GSH [73, 75]. As explained below, our previous studies and the data presented in this paper confirm the ability of GSH to dictate the formation of ZnS NCs [73]. We have previously reported that Zn–GSH complexes reacted with inorganic sulfide at room temperature to produce GSH-capped ZnS NCs [73]. Later studies on the synthesis of cysteine-capped ZnS NCs showed a positive influence of higher incubation temperatures on increased yield of NCs [75]. Cysteine proved superior to GSH in terms of the range of size distribution. However, the influence of temperature and the effects of ethanol precipitation had not been studied on the formation of GSH-capped ZnS NCs. Consequently, in this study we set out to determine the influence of temperature and precipitation on the formation of ZnS NCs capped with GSH.

UV/Vis spectra of freshly prepared samples of GSH–ZnS (Zn:S, 1:1) showed prominent peaks at 268 and 290 nm. The intensity of these peaks increased with time at 35 °C (figure 1(a)). In addition, the width of the peak narrowed suggesting decreased heterogeneity and nucleation to a discrete size range. Further incubation at 35 °C led to blue-shifting of the peaks with the conversion of the original peak at 290 nm to a peak at 278 nm and from 268 nm to a shoulder at 254 nm. Slightly different results were observed for the ZnS–GSH sample with a Zn:sulfide ratio of 1:1.5 (figure 1(b)). In this case, the less prominent peak at 268 nm completely disappeared. Furthermore, no blue shifting was observed. Curiously, this dual-peak phenomenon was only observed at 1.0 and 1.5 sulfide titrations. A single peak was observed at higher sulfide concentrations with or without heat treatment.

In a second synthesis procedure, ZnS–GSH was incubated for 30 min at room temperature (~25 °C) followed by incubation for an additional 30 min at 37 °C. This technique yielded ZnS–GSH that was characterized by a single peak between 275–290 nm depending on the amount of sulfide titrated. The remainder of the experiments was carried out using the second technique that reproducibly gave a single peak in the 275–290 nm range depending upon the amount of sulfide incorporated.
The phenomenon of dual peak formation and changes in the absorption spectra, particularly the blue shift in the absorption peak require further analyses. It is possible that such changes reflect variation in the crystal structure and/or change in size distribution. However, additional electron microscopic and x-ray diffraction data need to be studied to arrive at a possible explanation of such phenomena.

3.2. Size-selective precipitation and size-distribution analyses

Our previous initial work showed that Zn–GSH reacted with inorganic sulfide at room temperature to form NCs that exhibited a wide distribution of sizes even at a single titration of sulfide [73]. Differences in size distribution were assessed by size-exclusion chromatography in combination with spectroscopy. Similar observations were made on CdS–GSH NCs [74, 77]. In conformity with the earlier observation [73], gel filtration analyses showed that GSH-capped ZnS NCs prepared without any size-selective precipitation procedures were heterogeneous in size and composition (figures 2–4). These differences in size and composition are inferred from continuously changing (i) Zn/sulfide/GSH ratios (figure 2), (ii) absorption spectral profile (figure 3) and (iii) fluorescence spectra (figure 4) along the gel filtration fractions. It is clearly evident from the data presented in figures 2(a)–(e) that the fractions resolved by gel filtration continuously change in their sulfide/Zn/GSH ratios indicating a changing chemical composition. Significant quantities of free Zn–GSH complexes (fractions 25–35) were seen in samples prepared with initial sulfide/Zn ratios of up to 0.5:1 (figures 2(a) and (b)). Quantities of free Zn–GSH complexes (devoid of sulfide) decreased with increasing sulfide/Zn ratios (figures 2(c)–(e)). The changes observed in the chemical composition are reflected in the optical spectra of the fractions separated by gel filtration. The sample synthesized with an...
initial sulfide/Zn ratio of 0.25:1.0 was resolved into fractions that showed absorption peaks varying from 247 to 257 nm (figure 3(a)). Similar results were observed in samples prepared with initial sulfide/Zn ratios varying up to 1.5:1 (figures 3(b)–(d)). However, the sample prepared with an initial sulfide/Zn ratio of 5.0:1 did not show a significant variation in the absorption spectra. Fluorescence spectra of gel filtration fractions presented variations that were similar to those observed in the UV/Vis spectra of the same fractions (figures 4(a)–(e)). Again, the sample with the highest initial sulfide/Zn ratio showed minimal variation in the fluorescent spectra (figure 4(e)).

These results are consistent with observations made previously on GSH-capped ZnS or CdS NCs prepared without ethanol precipitation procedures [73, 74]. However, these observations contrast with cysteine-mediated or PC-mediated synthesis of NCs [74–76]. Cysteine- or PC-mediated synthesis resulted in more uniformly sized NCs as was determined by constant UV/Vis absorption spectral profiles along the gel filtration column [74–76]. The reasons for using gel filtration chromatography for size distribution analyses have been explained in detail elsewhere [60].

The ability of cysteine and PCs to dictate synthesis of significantly less heterogeneous CdS or ZnS NCs has been attributed to the higher stability constants of metal–cysteine or metal–PC complexes [87]. A logical explanation for these previous observations is that more stable metal-cysteine or metal-PC complexes restricted the incorporation of sulfide and consequently resulted in the formation of only discrete sizes of NCs [77]. The size-heterogeneity problem in the case of GSH-capped CdS complexes could be solved by selective ethanol precipitation in the presence of Na+ ions [77]. Furthermore, this procedure proved useful in removing unreacted Cd–GSH [77]. Similar procedures have been used in the present studies to achieve homogeneity of ZnS–GSH NCs in both size and chemical composition.

Ethanol precipitation did result in the isolation of ZnS–GSH that exhibited a narrow size-distribution range as determined by a combination of gel filtration and optical spectral analyses. Zn–GSH samples were reacted with inorganic sulfide to obtain sulfide/Zn ratios of 0.25, 0.5, 1.0, 1.5 and 5.0. The degassed samples were chromatographed on Sephadex G-25 to determine the composition of NCs in individual fractions (figures 5(a)–(e)). Gel filtration profiles showed predominantly a single peak containing Zn, GSH and sulfide in all cases, although the peak appeared to trail at lower sulfide/Zn ratios (figures 5(a)). It may have been due to incomplete removal of unreacted Zn–GSH at lower sulfide/Zn ratios. A small amount of free GSH could be seen in the sample prepared with 0.25 equivalents of sulfide to 1.0 of Zn (figures 5(a)). In contrast to our previous and current studies on unprecipitated samples (figures 2–4), GSH-capped ZnS NCs prepared by ethanol precipitation showed less significant changes in the ratios of various components of NCs.

The UV/Vis and fluorescence spectra of fractions from ethanol precipitated samples are shown in figures 6 and 7. Qualitatively similar spectra in adjacent fractions from a size-permeation column suggested a narrow size distribution in the original sample [76]. For each of the sulfide/Zn ratios, the UV/Vis spectra are overlapping, suggesting a narrow size distribution (figure 6). Furthermore, the size variation appears to be negligible between NCs prepared using sulfide/Zn ratios varying from 0.25:1 to 1.5:1. The typical absorption shoulder for the 1.5 sulfide sample (sulfide/Zn ratio of 1.5:1.0) was at 278 nm, whereas that for the 5.0 sulfide sample was red-shifted only by 17 nm to 295 nm. Fluorescence spectra corroborate the results obtained using UV/Vis spectroscopy (figures 7(a)–(e)). The present observations on very small differences between the
sizes of ZnS NCs prepared using varying initial sulfide/Zn ratios contrast with earlier observations on GSH-capped ZnS samples [73].

The striking differences between unprecipitated and precipitated samples could be attributed to effects of Na+ and ethanol treatment. Ethanol precipitation was shown to cause redistribution of the capping material and thus the formation of new NCs that appeared to exhibit very similar chemical composition [75–77]. This process appeared to occur irrespective of the capping material or whether the NC involved was ZnS or CdS [75–77]. It is also conceivable that the presence of free Zn–GSH in non-precipitated samples will influence the nature of ZnS NCs that were recovered following precipitation.

3.3. HRTEM, SAED and x-ray diffraction characterization of ZnS–GSH

In addition to spectroscopy and gel filtration experiments, HRTEM and electron diffraction were performed to account for the structure and size of ZnS–GSH NCs. The results obtained from the sample prepared with an initial sulfide/Zn ratio of 1:1 are presented (figure 8). Calculations based on effective mass approximation suggested an average size of 2.4 nm for ZnS showing an absorption peak at 275 nm [44,88]. However, the average size of ZnS–GSH NCs found was 3.45 ± 0.5 nm (mean ± s.e.m.) as shown in figure 8. Sooklal and co-workers have recently shown that effective mass approximation significantly underestimated the size of ZnS NCs [94]. Due to the small size of the crystals, the SAED patterns resembled ring polycrystalline patterns. The three strong reflections correspond to a hexagonal structure with \(d\)-spacings of 3.1, 1.9 and 1.62 Å (data not shown). This also correlates with the lattice fringes measurements and XRD results. The x-ray diffractogram (figure 9) with broad peaks at 3.12, 1.90 and 1.63 Å corresponds to hexagonal ZnS (10H). It is interesting to note that the observed \(d\)-spacings could be attributed to another hexagonal structure of ZnS (2H) which might indicate the presence of a mixture of ZnS (10H) and ZnS (2H). Alternatively, a hexagonal polymer of ZnS.

Figure 10. Effect of pH on the photodegradation of 4-nitrophenol (PNP) with ZnS–GSH. Degradation patterns obtained with Zn:sulfide ratios of 1:0.25, 1:0.5, 1:1.0, 1:1.5 and 1:5.0 are shown in (a)–(e), respectively. Labelling of the degradation profiles is shown in (b).

Figure 11. Absorbance at 405 nm as a function of time for solutions of 4-nitrophenol (PNP) with ZnS–GSH at different pH levels. The absorbance peaks correspond to the degradation of PNP, and the time taken for the absorbance to reach 0.5 is used to determine the degradation rate. The degradation rates are shown in (a)–(e), respectively. Labelling of the degradation profiles is shown in (b).
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Figure 11. Influence of the cap content of ZnS nanocrystals and the substrate:catalyst ratio on degradation. (a) A comparison of the effect of differently capped ZnS–GSH nanocrystallites on the photodegradation of PNP. The numbers 0.25, 0.5 etc indicate the initial sulfide/Zn ratios used in the preparation of the nanocrystals. (b) Activity of the catalyst at different catalyst:substrate concentrations. The ratios 2.5:1 etc refer to the ratio of the catalyst ZnS to substrate PNP. Both experiments were performed at pH 9.

with random stacking along the c-axis might also be present. Such random stacking along the c-axis is consistent with the observed broadening of the x-ray peaks and diffusiveness of the electron diffraction reflections which suggest a certain degree of disorder. The other peaks present in the x-ray diffractogram are consistent with GSH.

3.4. ZnS–GSH catalyzed photodegradation of PNP

The ability of GSH-capped CdS or ZnS NCs to photo-reduce various dyes has been demonstrated in previous studies [73–77]. Another major interest in the use of bulk or nano-semiconductors is in the area of water treatment employing procedures similar to advanced oxidation processes [37–41, 80, 81]. Both TiO₂ and CdS have been explored for photodegradation of organics in wastewater [37–41, 80, 81]. The use of CdS in such studies is impractical owing to the toxicity of cadmium [73]. A large number of publications have recently appeared on heterogeneous photocatalysis by TiO₂ [3, 8, 37–41, 80, 81, 93]. There have been relatively few studies on the use of ZnS [89–91]. ZnS NCs have the advantage of carrying out homogeneous catalysis, although UV illumination is needed for this class of NCs as well. In addition, heterogeneous catalysis with TiO₂ requires removal of this semiconductor at the end of the process. Zinc, being relatively nontoxic, need not necessarily be removed from the reaction vessel. The methodology described in this paper and our previous publications allows the synthesis of ZnS NCs with varying capping materials. The differences in the
nature and extent of capping will allow for the selection of most suitable NCs for such applications. It may also be noted that the size of ZnS particles capped with any of the biomolecules discussed here is approximately up to 5 nm based on effective mass approximation and direct electron microscopic measurements. These NCs thus have an additional advantage over ~30–50 nm TiO$_2$ particles due to significant increases in surface area.

We have PNP as a test compound to optimize various parameters to obtain maximal photodegradation. In the first of these experiments, NCs prepared using sulfide/Zn ratios varying from 0.25 to 5.0 were used at pHs 8, 9, 10 and 11 (figure 10). The sample with the least cap content (0.25 sulfide) exhibited very similar photoactivity at pH 8, 9 or 10. A considerable reduction in photodegradation occurred at pH 11. Somewhat similar results were obtained with ZnS NCs prepared using initially higher sulfide/Zn ratios (figure 10). Samples with lower cap content (i.e. initially higher sulfide/Zn ratios) showed slightly better results at pH 9, although rates were higher at early time-points for pH 8.0. It is evident from figure 10 that the least capped NC (with an initial sulfide/Zn ratio of 5:1) showed maximal activity. This finding was reconfirmed in a separate experiment showing that decreases in photoactivity were directly related to cap content (figure 11(a)). As mentioned earlier, GSH-capped ZnS NCs prepared by ethanol precipitation did not show as large variations in the cap content as was shown by GSH–ZnS NCs prepared without the ethanol fractionation procedure (figures 2 and 5).

It was hypothesized that one of the reasons for cap-dependent decrease in the photoactivity of GSH-capped ZnS NCs could be related to the ability of GSH to scavenge radicals [92]. Photodegradation of PNP was therefore tested in the presence or absence of both reduced and oxidized GSH (figure 12(a)). Interestingly, both oxidized and reduced GSH inhibited the photodegradation of PNP (figure 12(b)). Effects of reduced GSH could be attributed to free radical scavenging activity. Inhibition of photodegradation by oxidized GSH is
perplexing. However, it is likely that even oxidized GSH is reduced by ZnS NCs.

Optimal degradation of PNP occurred with PNP:ZnS-GSH concentrations (figure 12(b)) at 250 and 500 nmoles ml$^{-1}$ of ZnS to 50 nmoles ml$^{-1}$ of PNP (ratios at 5:1 and 10:1, respectively). Increasing the amount of the catalyst led to an apparent decrease in the velocity of the reaction. This was especially noticeable in the lag phase (figure 12(b)). This could be due to an inner-filter effect that reduces the amount of UV received by the catalyst. However, the degradation time seemed not to be affected, as observed in figure 12(b).

4. Conclusions

The present results in conjunction with our previous studies suggest novel means to produce uniformly sized NCs capped with cysteine or cysteine-containing peptides. Ethanol precipitation procedures further improve the isolation of NCs that are uniform in size as well as chemical composition. Surface characteristics of these NCs are dictated by the nature and extent of the capping material. In addition, unique metal–thiolate chemistry allows facile interchange of capping material even after it has been synthesized. These materials thus present interesting reagents to study the role of surface cap on the optical, electronic and other properties of NCs. We have specifically focused on the role of capping material in free-radical mediated reactions catalyzed by NCs upon irradiation. The ability of ZnS NCs to mediate rapid catalysis of PNP merits further investigations on a commercial level. Our experiments (to be described elsewhere) demonstrate that ZnS NCs are significantly superior to currently investigated TiO$_2$ in photodegradation experiments.

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