

Optimization of Dye-Doped Silica Nanoparticles Prepared Using a Reverse Microemulsion Method

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Received April 3, 2004. In Final Form: June 13, 2004

Fluorescent labeling based on silica nanoparticles facilitates unique applications in bioanalysis and bioseparation. Dye-doped silica nanoparticles have significant advantages over single-dye labeling in signal amplification, photostability and surface modification for various biological applications. We have studied the formation of tris(2,2'-bipyridyl)dichlororuthenium(II) (Ru(bpy)) dye-doped silica nanoparticles by ammonia-catalyzed hydrolysis of tetraethyl orthosilicate (TEOS) in water-in-oil microemulsion. The fluorescence spectra, particle size, and size distribution of Ru(bpy) dye-doped silica nanoparticles were examined as a function of reactant concentrations (TEOS and ammonium hydroxide), nature of surfactant molecules, and molar ratios of water to surfactant (R) and cosurfactant to surfactant (p). The particle size and fluorescence spectra were dependent upon the type of microemulsion system chosen. The particle size was found to decrease with an increase in concentration of ammonium hydroxide and increase in water to surfactant molar ratio (R) and cosurfactant to surfactant molar ratio (p). This optimization study of the preparation of dye-doped silica nanoparticles provides a fundamental knowledge of the synthesis and optical properties of Ru(bpy) dye-doped silica nanoparticles. With this information, these nanoparticles can be easily manipulated, with regard to particle size and size distribution, and bioconjugated as needed for bioanalysis and bioseparation applications.

Introduction

Nanoparticle-based bionanotechnology is a rapidly growing field that deals with particulate systems for bioanalytical, biotechnological, and biomedical applications. Nanoparticles (NPs) show many unique properties, such as optical, electrical, magnetic, and chemical, that are not observed in the bulk material. Dye-doped silica nanoparticles have significant advantages over single dye labeling in bioanalysis and biotechnological applications. They are made of thousands of organic or inorganic dye molecules doped inside a silica or polymer matrix. Incorporation of dye molecules inside the silica matrix protects the dye from the surrounding environment, increases photostability, and provides signal enhancement due to an increase in the number of dye molecules per nanoparticle.¹ These dye-doped nanoparticles are extensively used in photonics materials,² in nonlinear optical materials,³ and in bioimaging and biochemical analysis applications.^{1,4}

Although incorporation of dye-doped polymer nanoparticles has been well established, dye doping inside silica matrix is a challenge. The hydrophilic environment of silica does not favor entrapment of hydrophobic dye molecules. To successfully entrap dye molecules inside a silica matrix, polar dye molecules should be used to increase the electrostatic attraction of the dye with the negatively charged silica matrix and the size of the dye molecules should be large to prevent dye leakage from pores of the

silica matrix. Dye molecules have been entrapped inside silica matrix either by attaching water soluble dextran molecule to the dye or by introducing a hydrophobic silica precursor during synthesis of silica nanoparticles.⁵ The dye-doped silica nanoparticles were first synthesized by Van Blaaderen et al.⁶ using the Stöber method via FITC dye molecule conjugation with (3-aminopropyl)trimethoxysilane. The main advantage of silica nanoparticles over polymer nanoparticles is the ease of introduction of functional groups such as amines, thiols, carboxyls, and methacrylate on the surface by modification of surface hydroxyl groups. The dye-doped silica nanoparticles are ideal for bioanalysis applications because they are chemically inert, not subjected to microbial attack, and susceptible to swelling or changes in the porosity of the silica matrix with change in pH. However, particles prepared using the Stöber method are usually polydispersed, and it is difficult to obtain particle sizes below 100 nm. Alternatively, nanoparticles prepared using the water-in-oil microemulsion (W/O) are monodispersed, and it is relatively easy to control the size.^{7,8}

Dye-doped nanoparticles prepared in this work were synthesized using a water-in-oil microemulsion method. Water-in-oil microemulsion can be defined as a thermodynamically stable, isotropic, and transparent solution of oil, water, and surfactant, wherein the water droplets are dispersed as nanosized liquid entities in a continuous domain of oil. The nanodroplets of water serve as a nanoreactor for the synthesis of these nanoparticles. This method has advantages in that it does not require extreme conditions of temperature and pressure and the particle size and shape can be simply controlled by varying

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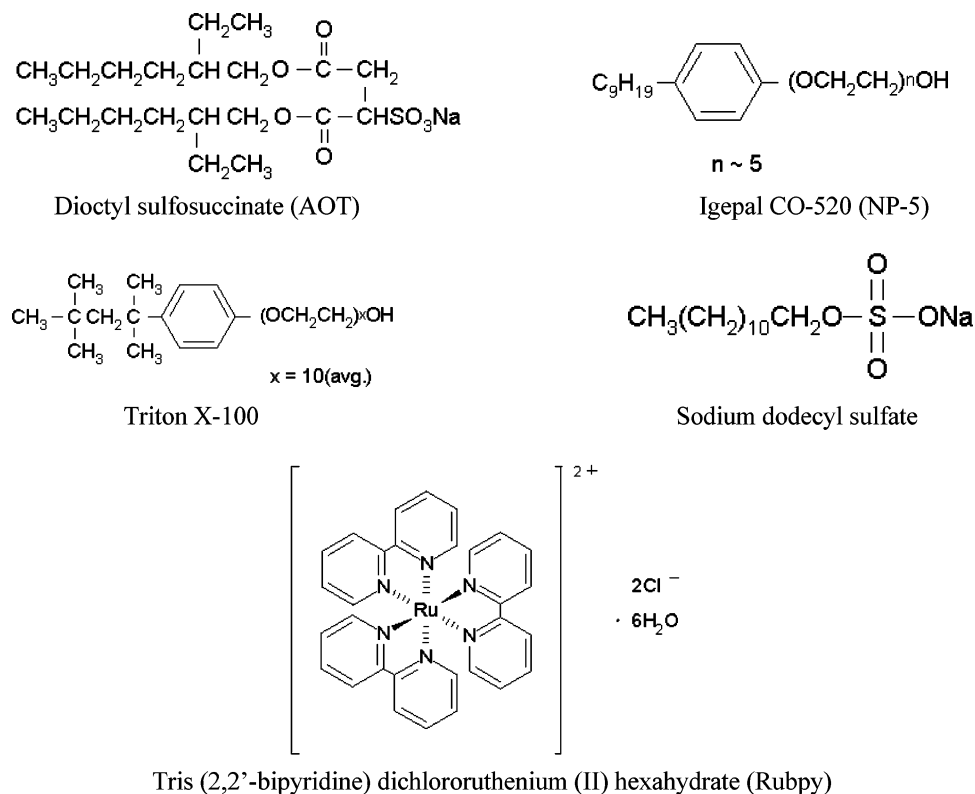


Figure 1. Structures of surfactants and dye molecules used in dye-doped silica nanoparticle synthesis.

microemulsion parameters.⁷⁻⁹ Various nanoparticles have been prepared¹⁰ using this method, showing its flexibility for the fabrication of different types and sizes of silica nanoparticles. Although Ru(bpy) dye-doped silica nanoparticles have been reported,¹ there were a few significant synthesis parameters that needed to be explored and better understood, such as the microemulsion characteristics: (1) the effect of molar ratios of water to surfactant and cosurfactant to surfactant, and (2) the nature of the surfactant molecule and reactant concentration effect on nucleation rate and dye location inside NP, as determined by the final particle size, polydispersity, and fluorescence spectra of doped dye molecules. These synthesis parameters need to be examined to further understand the formation of Ru(bpy) dye-doped silica nanoparticles in microemulsion and establish a scientific basis for synthesizing dye-doped silica nanoparticles with controlled size and fluorescence properties. A fundamental understanding of these parameters will provide a solid foundation for the effective application of these nanoparticles in bioanalysis and bioseparation applications.

In this study, we report the effect of the nature of surfactant molecules, the concentrations of tetraethyl orthosilicate and ammonium hydroxide, the water to surfactant molar ratio, and cosurfactant to surfactant molar ratio on the particle size, polydispersity, and fluorescence spectra of Ru(bpy) dye-doped silica nanoparticles.

Experimental Section

Materials. Surfactants. Commercial Triton X-100, sodium dodecyl sulfate (SDS), Aerosol OT or AOT (dioctyl sulfosuccinate) from Sigma, and Igepal CO-520 or NP-5 (polyoxyethylene nonyl phenol ether) from Aldrich were used for preparation of W/O microemulsion. The structures of the surfactants are shown in

Figure 1. Inorganic dye, Tris(2,2'-bipyridyl)dichlororuthenium(II) (Ru(bpy)), was purchased from Aldrich. Tetraethyl orthosilicate (TEOS) ($\text{Si}(\text{OC}_2\text{H}_5)_4$) with 99.999% purity, reagent grade heptane, cyclohexane, hexanol, and aqueous ammonia (NH_4OH) solution (71 wt % water, 29 wt % ammonia), all purchased from Aldrich, were used without further purification.

Synthesis. The nanoparticles were prepared using the microemulsion method. The microemulsion solution was prepared by mixing adequate amounts of surfactant, cosurfactant, organic solvent, aqueous solution of dye, water, and aqueous ammonia. Aqueous ammonia acts as both a reactant (H_2O) and a catalyst (NH_3) for the hydrolysis of TEOS. The volume of each microemulsion component varied with the type of surfactant used. In a typical microemulsion for preparation of silica nanoparticles using a ternary microemulsion system, 0.1 M surfactant was dissolved in 10 mL of heptane, followed by addition of 90 μL of aqueous solution of 0.1 M Ru(bpy) dye, 100 μL of TEOS, and 60 μL of 29.6 wt % NH_4OH . The reaction was allowed to stir for 24 h followed by addition of ethanol to break the microemulsion and recover the particles. The particles were washed a couple of times with ethanol and final washing with water. For silica nanoparticles, preparation in quaternary microemulsion using Triton X-100 as surfactant, the procedure consisted of mixing 1.77 g of Triton X-100, 1.6 mL of hexanol, 7.5 mL of cyclohexane, followed by addition of 400 μL of water, 80 μL of 0.1 M Ru(bpy) dye, 100 μL of TEOS, and 60 μL of NH_4OH . The reaction was allowed to stir for 24 h followed by addition of ethanol to break the microemulsion and recover the particles. The particles were washed two times with ethanol and one time with water.

Characterization. Transmission Electron Microscope Analysis. The size and morphology of dye-doped silica nanoparticles synthesized in W/O microemulsion were measured using a transmission electron microscope (Hitachi H-7000). The sample for transmission electron microscopy (TEM) was prepared by placing a few drops of the nanoparticle-containing microemulsion on 200 mesh carbon-coated copper grids. After evaporation of the solvent the particles were observed at an operating voltage of 200 kV. TEM micrographs of dye-doped silica nanoparticles were converted to digitized images from which diameters of about 200 particles were measured using imaging software (UTHSCA's Image tool).

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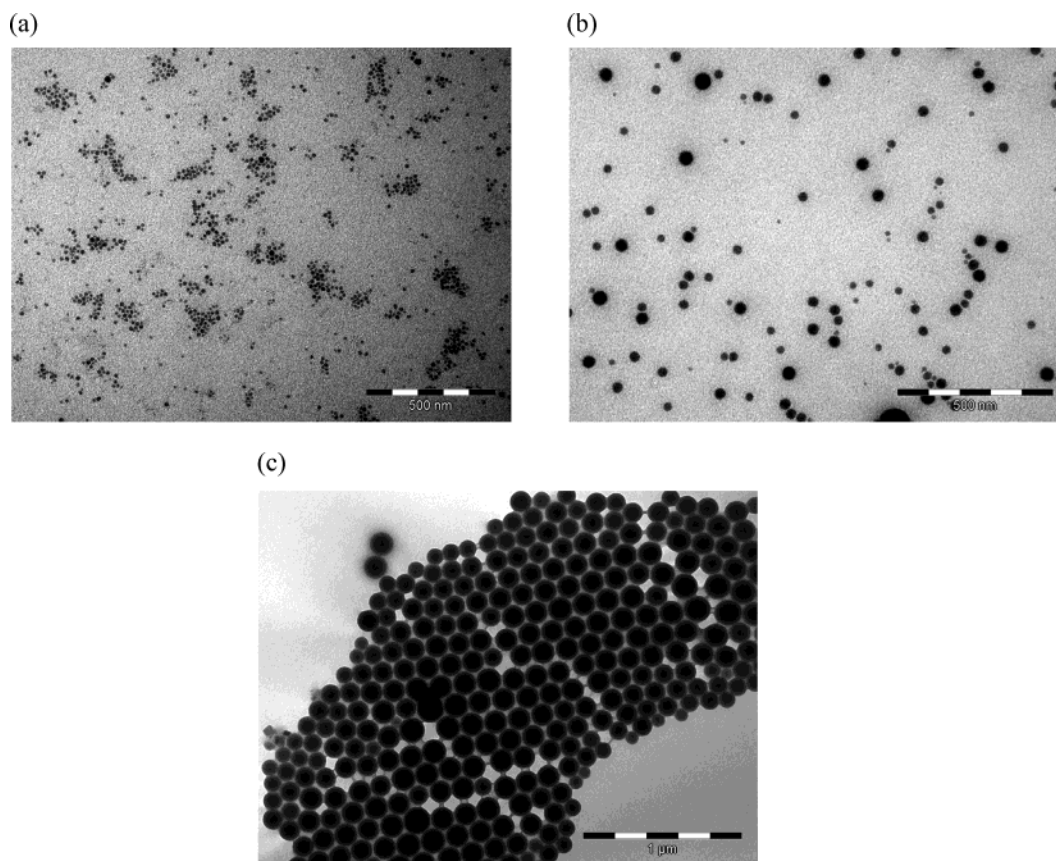


Figure 2. TEM micrograph showing the Ru(bpy) dye doped silica nanoparticles prepared in different microemulsion systems: (a) NP-5/cyclohexane/water; (b) AOT/heptane/water; (c) mixture, AOT + NP-5/heptane/water (The bar represents 1 μm in (a) and (b) 500 nm in (c).)

Table 1. Effect of the Nature of the Surfactant on Particle Size and Fluorescence Spectra of Dye-Doped Silica Nanoparticles Prepared in Different Microemulsions^a

microemulsion system	emission wavelength (nm)	max fluorescence intensity	particle size (nm) (TEM)	std dev
AOT/heptane/ water	595	2.7×10^6	29	8
AOT + NP-5/heptane/ water	597	2.1×10^6	130	8
NP-5/cyclohexane/water	588	1.7×10^6	14	2

^a For all microemulsions: water to surfactant molar ratio (R) = 10.

Absorbance and Fluorescence Studies. Absorbance and fluorescence measurements were performed by dispersing 1 mg/mL of the dye-doped nanoparticles in water and then observing the absorbance spectra on Varian Cary 100 spectrophotometer and fluorescence spectra using a Fluorolog Tau-3 spectrofluorometer (Jobin Yvon Spex Instruments, S.A. Inc.)

Results and Discussion

Effect of the Nature of the Surfactant. Figure 2 shows a TEM micrograph of Ru(bpy) dye-doped silica nanoparticles prepared in ternary microemulsions with different surfactants: anionic, AOT, nonionic, NP-5, and a mixture of AOT and NP-5. The particles were formed using the same reactant concentrations and water to surfactant molar ratio (R = 10). The total surfactant concentration was kept at 0.1 M for all three cases studied. The particles were spherical in shape, and the particle size was larger in microemulsions with NP-5 < AOT < AOT + NP-5. The number average particle size in the AOT microemulsion was 30 nm (standard deviation, δ = 8 nm), whereas with NP-5, it was 14 nm (standard deviation, δ = 2 nm) as shown in Table 1. The mixture of AOT and NP-5 in heptane resulted in the highest particle size of 130 nm (standard deviation, δ = 8 nm). These

variations in particle size are due to the structure of the microemulsion droplet as shown in Figure 3. AOT is a double-tailed anionic surfactant and has wedge-shaped geometry with a headgroup area smaller than the volume of the hydrocarbon tail. This geometry is suitable for the formation of reverse micelles having spherical droplet structure. A spherical geometry has been found in the entire single phase microemulsion zone of the phase diagram for this AOT-based microemulsion system, at different temperatures and with using various organic solvents.¹¹

In the case of microemulsion formed using NP-5 surfactant, at a water to surfactant molar ratio around 10 and higher, the microemulsion had a lamellar structure, wherein the water droplets tended to associate together and form interdroplet water channels, as shown using electrical conductivity and light scattering studies by Chang and Fogler.¹² Therefore, silica reacting species in NP-5 microemulsion are significantly less compartmentalized over droplets than those in AOT, leading to

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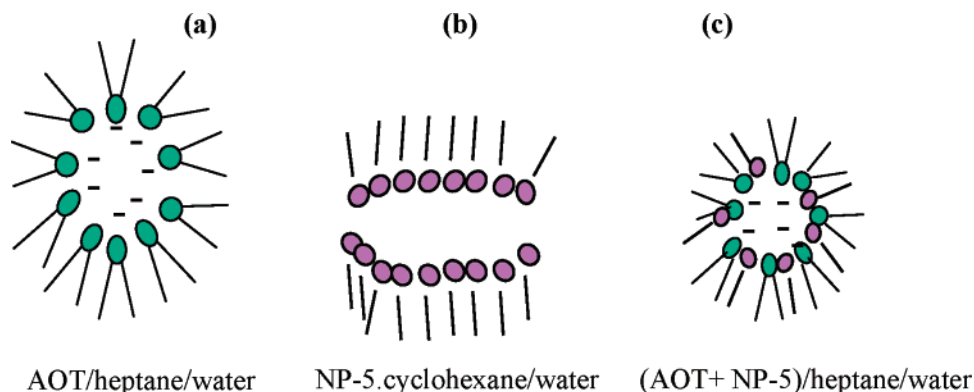


Figure 3. Structure of different microemulsions: (a) AOT/heptane/water; (b) NP-5/cyclohexane/water; (c) (AOT + NP-5)/heptane/water.

comparatively faster nucleation rate and smaller sized nanoparticles in NP-5 microemulsion. With the double-tailed surfactant and wedge-shape geometry, the AOT surfactant provides stronger steric film barriers to the interdroplet exchange of silica reacting species than those of NP-5. Consequently, fewer nuclei are formed and lead to larger particles.

Addition of NP-5 surfactant to the AOT microemulsion resulted in larger, monodisperse particles size with a diameter of 130 nm. This observation is contradictory to particle size trend for silver⁸ and MoS_x¹³ nanoparticles prepared in the same microemulsion system. The size of the silver nanoparticles was reduced from 22 to 7 nm, and the size of MoS_x reduced from 8 to 4 nm, when NP-5 was added to AOT/heptane microemulsion. In the latter case, the particles were prepared by mixing of two reactants in two different microemulsions, and nucleation and growth of particles were dictated only by the intermicellar exchange rate. For particles prepared by the direct addition of a reactant (e.g., TEOS) to the microemulsion containing ammonium hydroxide, in addition to intermicellar exchange rate, the diffusion of the reactants inside the micellar core plays a very important role. NP-5 acts as a cosurfactant causing a decrease in interfacial rigidity as a result of shielding of the negative charge between two anionic AOT surfactant molecules and increase in hydrophobic interaction between nonpolar tails of AOT and NP-5 (Figure 3c). This results in a decrease in microemulsion size,¹³ thus leading to more compartmentalization of silica reacting species and lower intermicelle exchange rate. These conditions lead to formation of fewer nuclei and thus a larger particle size.

Figure 4 shows the representative absorption spectra of Ru(bpy) dye doped silica nanoparticles. The absorption maximum is found to be at 454 nm. Figure 5 shows the emission spectra of Ru(bpy) dye doped nanoparticles at excitation wavelength of 450 nm. Further, Table 1 shows that the emission wavelength of Ru(bpy) dye doped nanoparticles was more red shifted in AOT and AOT + NP-5 than in NP-5 alone. The fluorescence spectrum of Ru(bpy) dye molecule was sensitive to the chemical environment around the dye molecule. Handa et al.¹⁴ studied the fluorescence spectra of Ru(bpy) dye in nonionic water-in-oil microemulsion and found two peaks corresponding to two different environments. The peak at shorter wavelength was due to dye molecules in less polar oxyethylene groups and the peak at longer wavelength

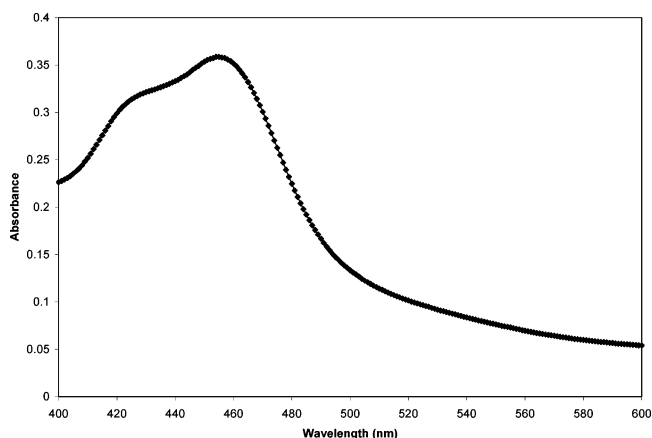


Figure 4. Absorption spectra of Ru(bpy) dye doped silica nanoparticles in water.

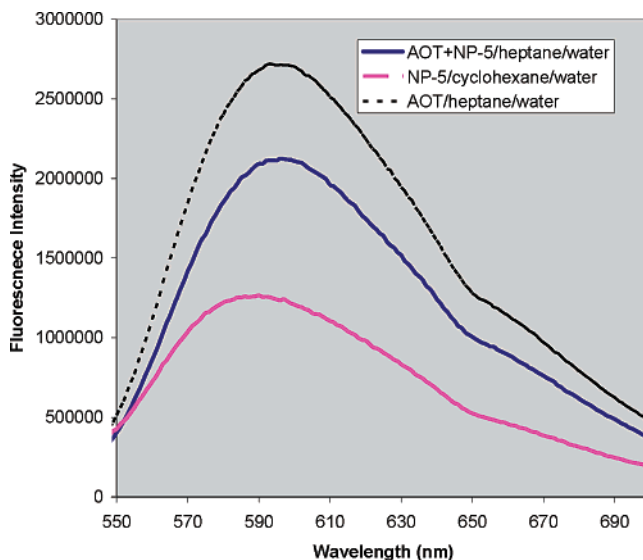


Figure 5. Effect of the nature of the surfactant molecules on the fluorescence spectra of Ru(bpy) dye doped silica nanoparticles. (Excitation wavelength = 454 nm.)

corresponded to dye molecules present in polar environment of water molecules present inside the reverse micellar core. Similarly, Arriagada and Osseo-Asare¹⁵ studied the fluorescence spectra of pure Ru(bpy) dye in water-in-oil microemulsion and found a red shift in fluorescence peak with an increase in the polarity sur-

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Table 2. Effect of Anionic Surfactant on Fluorescence Emission of Ru(bpy) Dye Doped Silica Nanoparticles Prepared in Triton X-100/Cyclohexane/Hexanol System

microemulsion system	emission wavelength (nm)	max fluorescence intensity
TX-100 + SDS/ hexanol/ cyclohexane	597	1.29×10^5
TX-100 + AOT/ hexanol/ cyclohexane	597	9.5×10^5
TX-100/ hexanol/ cyclohexane	590	11×10^6

rounding the probe molecule. The emission spectrum of pure Ru(bpy) dye in aqueous solution was 603 nm. The red shift in the fluorescence emission of Ru(bpy) dye doped silica nanoparticles, shown in Table 1, can be explained due to presence of anionic surfactant, which carries a negative charge. Ru(bpy) dye has a positive charge, and hence, the dye is closer to the microemulsion interface where the surfactant is present during the formation of particles. The dye molecules are in a more polar surrounding as compared to the nonionic surfactant, NP-5, wherein the dye molecules mostly reside in the bulk of silica matrix. Hence, when these dye-doped nanoparticles are dispersed in water, they have less interaction with the surrounding water molecules and show fluorescence emission at a lower wavelength relatively (see Figure 5, Table 1).

The red shift in fluorescence emission maxima was verified by adding anionic surfactants AOT or sodium dodecyl sulfate (SDS) to particles prepared in the Triton X-100/cyclohexane/hexanol/water system (Table 2). In both cases, a red shift was observed as compared to using the Triton X-100/cyclohexane/hexanol system alone, due to the presence of dye molecules close to the surface of the nanoparticle, where there is maximum interaction with the polar water molecules.

Effect of the Concentration of Reactants (Tetraethyl Orthosilicate (TEOS) and Ammonium Hydroxide). Formation of dye-doped silica nanoparticles inside W/O microemulsions takes place by hydrolysis of TEOS molecules using ammonium hydroxide as the catalyst. There are four main steps in particle formation: (1) association of TEOS with the W/O microemulsion; (2) TEOS hydrolysis and formation of monomers; (3) nucleation; (4) particle growth. After addition of TEOS molecules to the W/O microemulsion, the TEOS molecules get partitioned between W/O microemulsion and bulk organic medium by formation of a monomer having one silanol group (i.e., $\text{Si}(\text{OR})_3\text{OH}$). Further, hydrolysis leads to the formation of silicic acid, with four silanol groups ($\text{Si}(\text{OH})_4$). All of these species are associated with W/O microemulsion and contribute to the nucleation and growth. Since ammonium hydroxide is polar, it is present in the aqueous cores of reverse micelles. In order for hydrolysis to take place, the TEOS molecules have to diffuse from the surrounding organic phase to the W/O microemulsion.^{15–17}

The effect of the concentration of TEOS and ammonium hydroxide was studied in the quaternary microemulsion of Triton X-100/cyclohexane/hexanol/water using $R = 10$ and $p = 5.5$. We found that the increase in concentration of TEOS from 0.025 to 0.1 mM, with a constant concentration of ammonium hydroxide of 1.0 wt %, does not change the particle size and polydispersity of silica nanoparticles prepared in a Triton X-100/cyclohexane/water system. The number average particle size was found to be 82 nm with standard deviation around 15 nm and

fluorescence emission maxima at 590 nm. The above observation is due to the fact that excess TEOS molecules remain in the unhydrolyzed state and are unable to partition into the microemulsion phase.

Figure 6 shows TEM micrographs of dye-doped silica nanoparticles with increasing concentration of ammonium hydroxide. Ammonium hydroxide acts as a catalyst, providing OH^- ions necessary for hydrolysis of TEOS. It can be seen from the micrograph that the particles are spherical in shape. The particle size decreases and the number density of particles increase with an increase in the concentration of ammonium hydroxide. The particle size and polydispersity decrease from 82 to 50 nm and from 13 to 4 nm, respectively (Table 4). The increase in OH^- ions causes an increase in the rate of hydrolysis of TEOS molecules, and hence a large number of monomers are produced. Intramicellar nucleation occurs when the number of monomers inside the microemulsion exceeds a critical number, typically 2.¹⁸ Due to faster hydrolysis rate with an increase in OH^- ions, the ion/monomer occupancy number per micelle is greater than the critical nucleation number, resulting in a larger number of nuclei. Further, faster hydrolysis of TEOS produces an increased amount of ethanol as a byproduct. Ethanol acts as a cosurfactant, increasing the fluidity of the interface and, thereby, increasing the intermicellar exchange rate. These conditions further enhance nuclei formation and lead to a smaller particle size. The resulting particle size is, thus, smaller, and particles are monodispersed with increasing amounts of ammonium hydroxide. A gradual red shift in the fluorescence spectra was also observed as we increase the concentration of ammonium hydroxide. The concentration of ammonium hydroxide changes the pH inside the aqueous core of microemulsion. Arriagada et al. studied the effect of NH_4OH on the phase behavior of a single phase microemulsion domain. The single phase microemulsion zone was found to decrease as the concentration of ammonium hydroxide increases. Ammonium hydroxide acts as a lyotropic salt, reducing mutual solubility between water and surfactant. With increase in concentration of NH_4OH , more and more surfactant molecules are getting replaced by NH_4OH as there is more interaction of OH^- ions with water molecule and hence Ru(bpy) dye molecules (as they are solubilized in water). Thus, the Ru(bpy) dye molecules are more on the surface of nanoparticles and after synthesizing and dispersing in water react with surrounding water molecules, leading to a red shift.

Effect of Water to Surfactant Molar Ratio (R). An increase in water to surfactant molar ratio of water-in-oil microemulsions changes three parameters. First, it increases the size of the water pool of the reverse micelles, thereby increasing the ratio of bulk water molecules to surfactant-bound (hydrated) water molecules. Second, the increase in the size of the water pool increases the monomer-occupancy number or number of monomers per unit microemulsion droplet. Third, with an increase in R , the intermicellar exchange rate increases due to a decrease in the rigidity of the surfactant film.¹⁶

Table 4 shows the effect of water to surfactant molar ratio on particle size and fluorescence emission spectra for Ru(bpy) dye doped silica nanoparticles prepared in Triton X-100/cyclohexane/water microemulsion. At $R = 5$ the average particle size was 178 nm and decreased gradually to 69 nm at $R = 15$ (Table 4). At low R values, the microemulsion droplet size is smaller, and hence the number of TEOS molecules partitioned at the interface of the surfactant and water is less. As a result, there is

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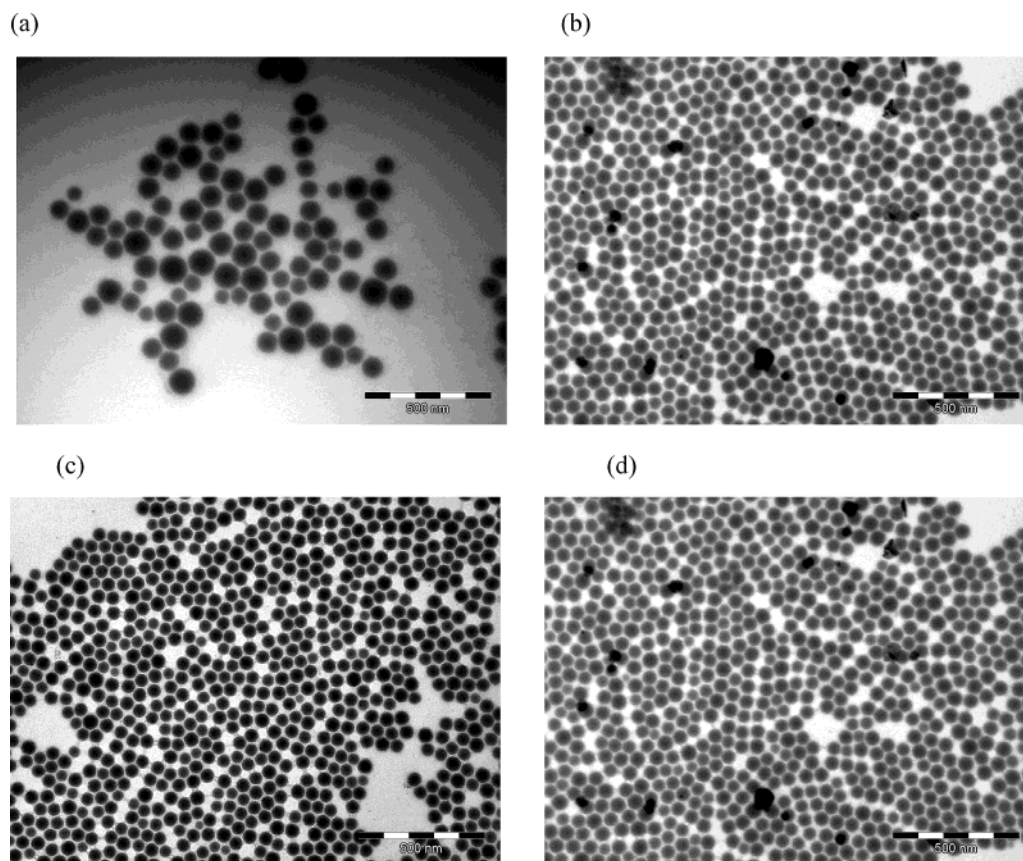


Figure 6. Transmission electron micrograph of Ru(bpy) dye doped silica nanoparticles prepared in Triton X-100/cyclohexane/hexanol microemulsion showing effect of ammonium hydroxide concentration: (a) 0.5 wt %; (b) 1.0 wt %; (c) 1.50 wt %; (d) 2.0 wt %. (The scale bar represents 500 nm.)

Table 3. Effect of Concentration of Ammonium Hydroxide on Particle Size, Standard Deviation, and Fluorescence Properties of Ru(bpy) Dye Doped Silica Nanoparticles in Triton X-100/Cyclohexane/Hexanol System

ammonium hydroxide (wt %)	emission wavelength (nm)	max fluorescence intensity	particle size (nm) (TEM)	std dev
0.5	590	11×10^6	82	13
1.0	591	9×10^6	54	5
1.5	594	8.7×10^6	52	6
2.0	596	5.9×10^6	50	4

Table 4. Effect of Water to Surfactant Molar Ratio on Particle Size and Fluorescence Spectra of Ru(bpy) Dye Doped Silica Nanoparticles Prepared in Triton X-100/Cyclohexane/Water System

water/surfactant molar ratio	emission wavelength (nm)	max fluorescence intensity	particle size (nm) (TEM)	std dev
5	594	1.6×10^7	178	29
10	591	1.1×10^7	82	14
15	590	1.5×10^7	69	4
20	587	1.3×10^7	-	-

a smaller number of monomers and nuclei formed. Further, conditions of bound water and rigid interface due to close packing of surfactant molecules make the mobility of these monomers less, decreasing the intermicellar exchange. These conditions make the environment conducive for less nuclei formation and a more enhanced growth rate. Hence the terminal particle size is larger. However, the number of monomers and oligomers available for growth are more, and hence the final particle size is larger. As the R value increases, the droplet size of the microemulsion increases and the amount of bulk

water molecules also increases. As a result, more of the TEOS molecules are hydrolyzed at the interface. Also the polarity of droplet and the partition coefficient of TEOS increase with size of droplet. Under these conditions, the rate of intramicellar nucleation increases and the resultant particle size is smaller. Furthermore, at $R = 15$ in addition to an increasing the droplet size and increasing the bulk water molecule, the intermicellar exchange causes further enhancement in the nucleation rate, further decreasing particle size and increasing the monodispersity of dye-doped silica nanoparticles.

There is a blue shift in the fluorescence spectra with an increase in the R value from 5 to 15. At $R = 5$, the droplet size is smaller and most of the water molecules are bound to the surfactant. Thus dye molecules under these conditions are mostly at the interface; hence, more interaction with the surrounding environment. With an increase in the water to surfactant molar ratio, the amount of bulk water molecule increases and causes the dye molecules to be more toward the bulk than at the interface, resulting in the blue shift of the spectra.

Effect of Molar Ratio of Cosurfactant to Surfactant (p). The molar ratio of cosurfactant to surfactant (p) was varied by changing the concentration of hexanol in the microemulsion system of Triton X-100/cyclohexane/water. Addition of medium-chained alcohol changes the microemulsion in two ways. First, an alcohol molecule adsorbs at the interfacial film of microemulsion droplets and thus alters the packing parameter of surfactant. This influences the radius of curvature of the droplet. Second, it decreases the interfacial rigidity causing more fluid interface and hence increase in intermicellar exchange.^{19,20}

Figure 7 shows TEM micrographs of Ru(bpy) dye doped silica nanoparticles with an increase in cosurfactant to

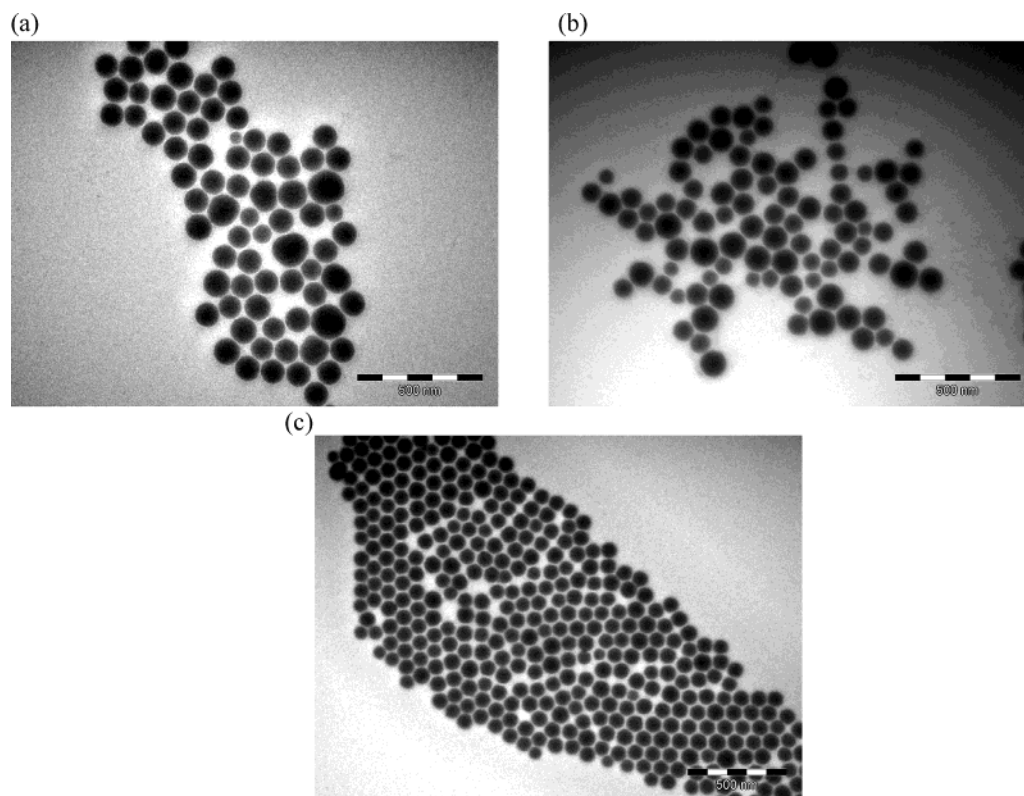


Figure 7. Transmission electron micrograph of Ru(bpy) dye doped silica nanoparticles showing effect of cosurfactant to surfactant molar ratio (p): (a) $p = 2.7$; (b) $p = 5.5$; (c) $p = 7.7$. (The bar represents 500 nm.)

Table 5. Effect of Cosurfactant to Surfactant Molar Ratio (p) on Particle Size and Fluorescence Spectra of Dye-Doped Silica Nanoparticles Prepared in Triton X-100/Cyclohexane/Water

cosurfactant to surfactant molar ratio	emission wavelength (nm)	max fluorescence intensity	particle size (nm) (TEM)	std dev
2.7	590	1.3×10^7	97	14
5.5	590	1.1×10^7	82	13
7.7	591	1.1×10^7	72	6
11	592	1.1×10^7		

surfactant molar ratio from 2.7 to 11. The particle size decreased as we increased the concentration of cosurfactant (Table 5). These observations are due to the increase in the flexibility of the interfacial film and increase in number of droplets with an increase in the concentration of cosurfactant. The increase in the number of droplets causes more TEOS molecules to be adsorbed at the oil–water interface than in the bulk. This adsorption leads to more partitioning of TEOS molecules, resulting in more nuclei formation inside the water-in-oil microemulsion droplets. Further, an increase in the fluidity of the interface causes more successful collisions between two droplets, thus favoring conditions for a large number of nuclei formation and decrease in growth rate of the nanoparticles.

Fluorescence emission spectra of Ru(bpy) dye doped inside silica nanoparticles did not show much change and

remained constant at 590 nm with a fluorescence intensity of 1.1×10^7 . Hence, the dye molecules are present in the same environment and cosurfactant has a negligible effect on emission properties of dye molecules.

Conclusions

We have optimized the preparation of dye-doped silica nanoparticles for bioanalysis and bioseparation applications. The following conclusions can be drawn from this work: (1) The fluorescence spectra and the particle size were strongly affected by the nature of the surfactants. The smallest and most monodispersed particle size obtained was 14 nm in the microemulsion system of NP-5/cyclohexane/water. The emission spectrum of Ru(bpy) had red shifted when an anionic surfactant is incorporated into the microemulsion. (2) An increase in the concentration of ammonium hydroxide increased the rate of nucleation, resulting in smaller nanoparticle size, monodisperse particles, and a red shift in the emission spectra of Ru(bpy). (3) The particle size was found to be a function of water to surfactant molar ratio and cosurfactant to surfactant molar ratio, both showing particle size increase with an increase in their respective ratios. This optimization study provides a solid foundation for the development and effective utilization of various sizes of dye-doped silica nanoparticles that can be used in unique bioanalysis and biotechnological applications.

Acknowledgment. This work was partially supported by a NIH R01 GM66137, NSF NIRT grant and by a Packard Foundation Science and Technology Award.

LA049137J

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