Surface Modification of Y_2O_3 Nanoparticles

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Rare earth ion-doped yttrium oxide (Y_2O_3) nanocrystals are nontoxic and can be prepared as upconversion materials for cellular imaging, but they do not suspend well in water. In contrast to their tendency to dissolve in acidic media, yttria (Y_2O_3) nanoparticles readily react with phosphonic acids to give phosphonate-bonded yttria particles. Through the choice of phosphonic acid, the hydrophilicity of the nanoparticles can be controlled. The synthesis of a novel tetraethylene glycol-derived phosphonic acid is described; yttria treated with the corresponding phosphonate is easily dispersed in aqueous media. The preparation of yttria bonded to a phosphonate that may be used for cross coupling with biomolecules is also described.

Introduction

Semiconductor quantum dots have received significant attention for biological applications such as cellular imaging,[1] but their constituent toxic elements (e.g., CdSe) and their need for UV excitation can limit their use in vitro and compromise in vivo applications.[2] Rare earth ion-doped yttrium oxide (Y_2O_3) nanocrystals are an interesting alternative to CdSe quantum dots for two significant reasons: they are nontoxic,[3] and they can be prepared as upconversion materials.[4] In the latter context, they absorb multiple infrared photons and emit in the visible region.[5] Because IR excitation is less damaging and penetrates further into living tissue than UV, upconverting yttria is a promising material for in vivo imaging.[6] However, untreated yttria nanocrystals are not without problems: particles tend to aggregate, and they lack surface groups that can be used to attach targeting biomolecules.[7] Whereas surface coating with silica/siloxane layers is a common method of enabling particle−biomolecule conjugation, this treatment can significantly increase particle size,[8] which affects transport to and into cells, and silica and siloxane coatings can be hydrolytically unstable under physiological conditions.[9] A surface treatment that yielded a robust, covalently bound, hydrolytically stable organic monolayer capable of being functionalized would seem to be superior. It would only nominally increase the particle size yet allow the particle to be chemically bound to a targeting reagent. We have shown that phosphonic acids can react with a variety of metal oxide surfaces to yield, ultimately, phosphonate monolayer films.[10] Given the tendency of yttria to dissolve in acidic solutions, we were surprised to discover that Y_2O_3 particles can react with phosphonic acids to give stable phosphonate-coated materials. Herein we show that, through the choice of phosphonic acid structure, these yttria particles can be made to be either more hydrophilic or less hydrophilic than the native oxide and can be activated for the covalent attachment of biomolecules.

Experimental Section

Acetoxy(tetra[ethyleneoxy])propyl Dimethyl Phosphonate (5). A modification of the literature procedure[11] was used. Dimethyl phosphite (2.481 g, 0.0226 mol) and **4** (2.095 g, 0.0076 mol) were added to a 50 mL three-necked flask fitted with a reflux condenser, an argon inlet, and a septum. The stirred solution was heated to 105 °C. *tert*-Butyl peroxybenzoate (0.1 mL, 5.26×10^{-4} mol) was added via syringe to initiate the radical addition. An additional portion of radical initiator was added after 1 h and again after 2 h of reaction time. After 4.5 h of total reaction time, the mixture was allowed to cool. The resulting oil was purified by silica column chromatography using increasing amounts (0−6%) of methanol in ethyl acetate to obtain a colorless oil (0.750 g, 25% yield). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 4.21 (dd, J = 3.9, 5.4 Hz, 2H), 3.74 (d, J = 10.7 Hz, 6H), 3.72−3.56 (m, 14H), 3.51 (t, J = 6 Hz, 2H), 2.06 (s, 3H), 1.92−1.76 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 171.16, 70.86, 70.72, 70.67, 70.21, 69.22, 63.71, 52.42 (d, J = 6 Hz), 22.77 (d, J = 5 Hz), 21.37 (d, J = 141 Hz), 21.09. ^{31}P NMR (121.6 MHz, $CDCl_3$ δ): 36.21. FTIR (neat): 3461, 2952, 2870, 1738, 1456, 1375, 1352, 1246, 1182, 1113, 1055, 1031, 952, 843, 810 cm^{-1}. LRMS (EI): 387 (M + H)

Hydroxyl(tetra[ethyleneoxy])propyl Phosphonic Acid (1b). This method was adapted from a literature procedure.[12] A solution of **5** (0.271 g, 7.01×10^{-4} mol) in 15 mL of CH_2Cl_2 was added to a 50 mL three-necked flask equipped with a stir bar, an argon inlet, and a septum. The solution was stirred at room temperature under argon, and trimethylsilyl bromide (0.5 mL, 3.79×10^{-3} mol) was added dropwise via syringe. The reaction mixture was stirred for 24 h. Methanol (0.109 g, 3.40×10^{-3} mol) was then added, and the solution was stirred for an additional 24 h. Hydrochloric acid (1 mL, 5% HCl solution in deionized water) was added, and the mixture was stirred for 30 min before the layers were allowed to settle. The aqueous layer was collected, and the water was evaporated under reduced pressure to yield an orange oil (0.232 g, 104%). The product was >95% pure by ^{31}P NMR spectroscopy, and no significant impurities were visible in the ^{1}H or ^{13}C NMR spectra so **1b** was not purified further before use (Scheme 1). It is likely that all of the water is not removed during evaporation because of the hydrophilic

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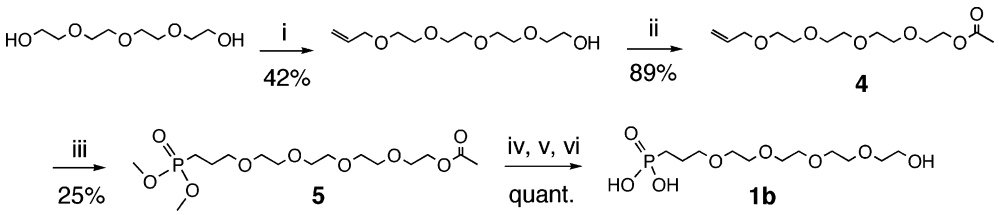
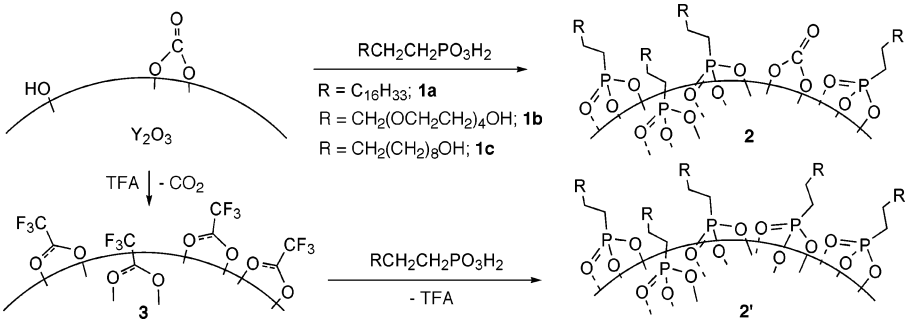
Scheme 1. Synthesis of Hydroxy(tetra[ethyleneoxy])propyl Phosphonic Acid (1b)[a]



[a] (i) Allyl bromide, NaOH, THF, reflux for 3 h; (ii) AcCl, DIPEA, CH_2Cl_2, 0 °C for 3 h; (iii) dimethyl phosphite, *tert*-butyl peroxybenzoate, 105 °C for 4.5 h; (iv) TMS−Br, CH_2Cl_2, 24 h; (v) MeOH, 24 h; (vi) HCl, 30 min.

Scheme 2. Surface Modification of Yttria Particles by Hydrophobic (2a) or Hydrophilic (2b) Phosphonates or by Phosphonates of Intermediate Hydrophilicity (2c)



nature of **1b**. ^{1}H NMR (300 MHz, CD_3OD, δ): 3.70−3.52 (m, 18H), 1.93−1.75 (m, 4H). ^{13}C NMR (125.7 MHz, CD_3OD, δ): 73.61, 71.88, 71.74, 71.62, 71.56, 71.42, 71.19, 62.29, 24.43 (d, J = 139 Hz), 24.13 (d, J = 4 Hz). ^{31}P NMR (121.6 MHz, CD_3OD, δ): 32.47. FTIR (neat): 3362, 2927, 2881, 1718, 1456, 1352, 1249, 1086, 946 cm^{-1}. LRMS (ESI): 315 (M − H)

Carbonate Removal by TFA (3). Yttria nanoparticles (∼35 mg, Nanocerox) were dispersed by sonication in 20 mL of acetonitrile and were transferred to a 50 mL round-bottomed flask. Trifluoroacetic acid (1.5 g) was added, and the reaction mixture was stirred overnight (15 h) at room temperature. Nanoparticles were collected by centrifugation, and the solvent was removed under reduced pressure to give **3**.

Phosphonate Film Formation. Nanoparticles (35 mg, as received or TFA-treated, **3**) were dispersed by sonication in 10 mL of THF and were transferred to a 50 mL round-bottomed flask containing the phosphonic acid (2.3×10^{-5} mol of **1a**, **1b**, or **1c**) in 25 mL of THF. The reaction mixture was then stirred for 3 h at room temperature, and the nanoparticles were collected by centrifugation. In the case of coating with **1a** (to give **2a** or **2a′**), 10 mL of methanol was added to the THF suspension to help precipitate the particles on centrifugation. The phosphonate-coated nanoparticles were washed by dispersing in 20 mL of methanol containing 9 drops of 0.05 M NaOH/methanol solution. This suspension was manually shaken for 5 min, and the particles were collected by centrifugation to yield **2a**, **2b**, or **2c** (Scheme 2).

Results and Discussion

Yttria reacts with ambient water[13] and CO_2,[14] and the IR spectrum of yttria nanoparticles is dominated by carbonate ($\nu_{CO_3^-}$ = 1530, 1405 cm^{-1}) and OH (ν_{OH} = 3400 cm^{-1}) bands (Figure 1); the latter band is attributed to both yttria surface hydroxyl groups and strongly adsorbed molecular water. High-temperature (900 °C) treatment removes virtually all of the surface water[13] and most of the carbonate,[14] but such harsh conditions can result in deleterious particle aggregation and sintering.[15] Thus, we were happy to find that, even in the presence of these surface contaminants, simply stirring a suspension of Y_2O_3 nanoparticles in a tetrahydrofuran (THF) solution of octadecylphosphonic acid (ODPA; **1a**) gave, after isolation and rinsing, particles coated with a film of octadecylphosphonate (**2a**). The IR spectrum (Figure 2) had peaks ν_{CH_2} = 2919 and 2850 cm^{-1} characteristic of ordered aliphatic chains.[16] There was also a broad band from 1160 to 990 cm^{-1} and a sharp peak at 1090 cm^{-1} attributed to ν_{P-O}; there was no band at 1240 cm^{-1} ($\nu_{P=O}$), which is consistent with a tridentate-bound phosphonate film.[17] However, carbonate bands were still apparent.

We find that surface carbonate can be removed from yttria by treating a suspension of the uncoated particles with trifluoroacetic acid (TFA) in acetonitrile. The resulting material (**3**) showed trifluoroacetate coordination ($\nu_{RCO_2^-}$ = 1675, 1460 cm^{-1}; νC−F = 1220, 1160 cm^{-1}, Figure 1) and was more resistant than native yttria to reaction with atmospheric CO_2. It did, however, react readily with a solution of **1a**: after rinsing with methanolic NaOH,

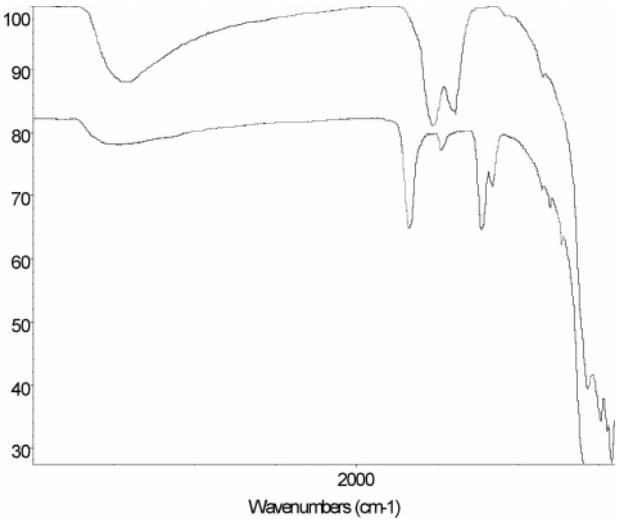


Figure 1. IR spectra of as-received Y_2O_3 (top) showing strong carbonate bands that can be removed by TFA treatment to yield **3** (bottom).

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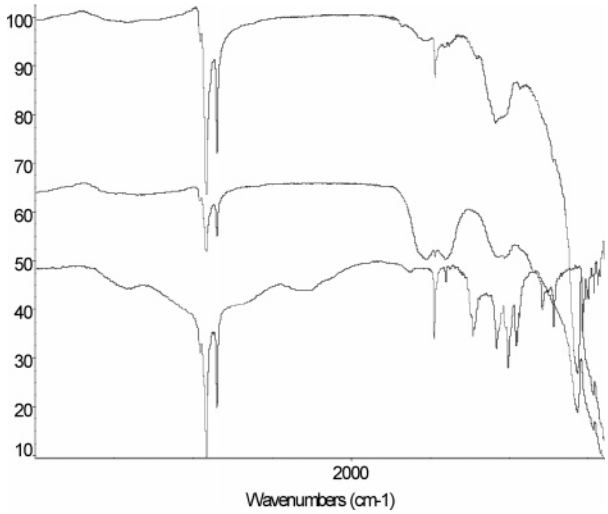

Figure 2. IR spectrum of **1a** (bottom), which was deposited on Y_2O_3 and **3** to yield **2a** (middle) and **2a′** (top), respectively.

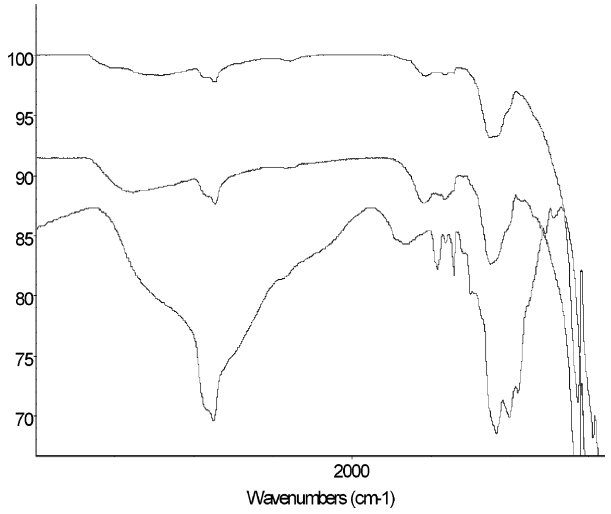

Figure 3. IR spectra of **1b** (bottom) and hydrophilic nanoparticles of **2b** (middle) and **2b′** (top).

the IR spectrum of these particles (**2a′**) showed the trifluoroacetate bands to be absent and aliphatic and phosphonate regions to be similar to those for **2a** (Figure 2). Apparently TFA treatment, although useful in removing surface carbonate, has only a small effect on the formation of the deposited organic film. It may be that, in the absence of TFA pretreatment, an ordered organic film exists in regions separated by carbonate islands.

As-received yttria nanoparticles do not suspend well in various organic solvents such as THF; they agglomerate even after sonication. SEM images of these particles show micrometer-dimension aggregates of 40−80 nm crystallites. Dynamic light scattering (DLS) measurements of nanoparticles dispersed by sonication in THF show an average hydrodynamic diameter of approximately 420 nm. It is likely that sonication fractures the micrometer-dimension aggregates seen by SEM into the smaller ones measured by DLS. Particles of **2a** and **2a′** sonicated in THF showed DLS particle sizes of 296 and 466 nm, respectively. The difference in measured particle sizes between **2a** and **2a′** may result from differences in their surface composition (surface carbonate and/or phosphonate) and solvents used in their preparation (**2a** in THF; **3**, the precursor of **2a′**, in acetonitrile before reaction with **1a**). Despite their relatively large sizes, the particles were easily suspended in nonaqueous media. Interestingly, gross suspendability characteristics did not depend on the presence of residual carbonate: both TFA-treated and non-TFA-treated **2a′** and **2a** showed suspendability that was good in $CHCl_3$ or CH_2Cl_2 and better in THF or ethyl acetate. Thus, whereas any remaining large aggregates quickly settled out, smaller particles remained suspended for weeks.

As-received yttria nanoparticles can be suspended in water with sonication, but they begin to agglomerate and settle out of suspension after several hours. Because biological applications might require better aqueous suspendability for these nanoparticles, we synthesized and bound a novel tetraethylene glycol-containing phosphonate to the yttria surface (Schemes 1 and 2). First, tetraethylene glycol was monoallylated[18] using allyl bromide and NaOH in refluxing THF and was then acetylated[19] in CH_2Cl_2 at 0 °C using acetyl chloride and a tertiary amine to give **4**. Dimethyl phosphonate ester derivative **5** was then prepared using 3:1 dimethyl phosphite:**4** heated to 105 °C.[3,11] Radical initiator *tert*-butyl peroxybenzoate was then added; additional *tert*-butyl peroxybenzoate was added after 1 and 2 h of reaction. Attempts to purify **5** by distillation failed, but chromatography on silica was adequate. The ^{1}H NMR spectrum of **5** consists of a series of complex multiplets at δ 1.95−1.78 for the methylene protons closest to the phosphorus ($^1J_{P-C}$ = 141 Hz; $^2J_{P-C}$, = 5 Hz) and a series of overlapping AA'BB' patterns for the CH_2 groups of the ethyleneoxy units (δ 4.2 and 3.8−3.5). Phosphonate ester groups of **5** (^{31}P NMR, $CDCl_3$, δ 36.21) were cleaved using trimethylsilyl bromide (4 equiv in CH_2Cl_2,) followed by treatment with methanol (24 h at room temperature).[12] Hydrolysis of the acetate with a stoichiometric amount of aqueous HCl gave hydroxy(tetra[ethyleneoxy])propylphosphonic acid (**1b**) (^{31}P NMR, CD_3OD, δ 32.47). The phosphonic acid was deposited on yttria or TFA-treated yttria (**3**) by stirring the particles for 3 h in a THF solution of **1b** to give **2b** or **2b′**, respectively (Scheme 1). Any impurities remaining from the preparation of **1b** were deemed to be removed by washing particles of **2b** and **2b′**. IR spectra of **2b** and **2b′** were similar, with the exception of carbonate bands present in **2b** (Figure 3). For both **2b** and **2b′**, ν_{CH_2} = 2870 (br) and 2930 cm^{-1} (shoulder) were observed, similar to disordered oligoethylene glycol chains of thiolate derivatives on gold.[20] Similar to **2a**, the band for ν_{P-O} from 1200 to 960 cm^{-1}, the peak at 1110 cm^{-1}, and the absence of a peak near 1240 cm^{-1} are consistent with tridentate phosphonate coordination.[17]

Particles of **2b** and **2b′** suspend readily in water with agitation. Sonication enables longer-term suspension. Smaller particles remain suspended for days, and unlike as-received particles that stay visibly aggregated after settling out, **2b** can be resuspended easily by manual agitation. Hydrodynamic diameters of as-received particles in water were measured to be 300 nm, whereas those of **2b** and **2b′** were 238 and 400 nm, respectively. Here, too, observed DLS size differences are likely derived from particle surface composition and the solvent employed in their preparation.

Partitioning of **2b** and **2b′** between $CHCl_3$ and water occurred as expected for the hydrophilicity of the phosphonate monolayer. Mixing $CHCl_3$ and hydrophilic **2b** or **2b′** followed by the addition of water and agitation gave an aqueous suspension of nanoparticles above the organic layer. Conversely, mixing water and hydro-

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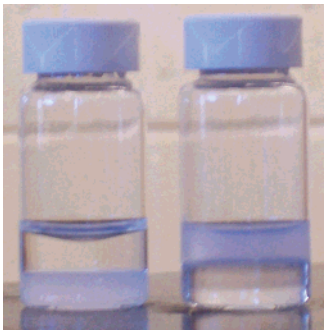

Figure 4. Partitioning of modified nanoparticles in biphasic $CHCl_3$/H_2O solvent mixtures. (Left) **2a** (hydrophobic, **1a**-coated) suspended in a $CHCl_3$ layer. (Right) **2b** (hydrophilic, **1b**-coated) suspended in a water layer.

phobic **2a** or **2a′** showed particles floating on the water surface until $CHCl_3$ was added and the mixture was sonicated. The biphasic mixture was then a suspension of **2a** or **2a′** in the organic layer only (Figure 4).

A third system was examined in which yttria nanoparticles were bonded to 11-hydroxyundecylphosphonic acid (**1c)**, which we have previously used to attach biomolecules to solid metal oxide surfaces.[10,21] The resulting particles have hydrophilicity intermediate between that of **2a** and **2b**. IR spectra are characteristic of less-well-ordered films with $\nu_{CH_2} = 2923$ and 2852 cm^{-1} for **2c** and 2921 and 2851 cm^{-1} for **2c′**. The phosphonate regions for **2c** and **2c′** showed ν_{P-O} as a broad band centered at 1060 cm^{-1}, similar to those for **2a** and **2b**.

Conclusions

We have shown that phosphonates readily bond to yttrium oxide nanoparticle surfaces and that the hydrophilic characteristics of these particles can be controlled by choice of the phosphonic acid. Bonding apparently occurs at room temperature, which contrasts with the reactivity of phosphonic acids with particles of several other metal oxides. For example, alkylphosphonic acids have been used to surface modify iron oxide nanoparticles, but this requires elevated temperature, long reaction times, and/or high-energy ultrasound treatment.[22,23] Phosphonic acid reactivity seems to correlate with the basicity of the metal oxide substrate; for example, Al_2O_3 and ZrO_2 show higher reactivity toward them than does TiO_2.[24] The ease with which yttria reacts with ambient CO_2 suggests a surface of high basicity;[25] however, unlike Al_2O_3, which is also highly reactive with phosphonic acids and gives phosphonate salts,[24] yttria yields a stable organic film. The coated particle aggregates described herein may be of immediate use for certain bioassays,[26−30] but in vivo applications may require smaller particles and/or aggregates. Studies using such smaller particles are commencing; others, to conjugate biomolecules to yttria particles using hydroxyl-terminated phosphonate films, are underway.

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Supporting Information Available: Preparation of tetraethylene glycol monoallyl ether and **4**. ^1H and ^{13}C NMR spectra of **1b** and **5**. IR spectra of **2c** and **2c′**. This material is available free of charge via the Internet at http://pubs.acs.org.

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