Published in final edited form as:

ACS Appl Mater Interfaces. 2018 February 07; 10(5): 4953–4960. doi:10.1021/acsami.7b15943.

Polystyrene Core, Silica Shell Scintillant Nanoparticles for Low-Energy Radionuclide Quantification in Aqueous Media

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Abstract

β -particle emitting radionuclides are useful molecular labels due to their abundance in biomolecules. Detection of β -emission from ^3H , ^{35}S , and ^{33}P , important biological isotopes, is challenging due to the low energies ($E_{\text{max}} \sim 300 \text{ keV}$) and short penetration depths ($\sim 0.6 \text{ mm}$) in aqueous media. The activity of biologically relevant β -emitters is usually measured in liquid scintillation cocktail (LSC), a mixture of energy-absorbing organic solvents, surfactants, and scintillant fluorophores, which places significant limitations on the ability to acquire time-resolved measurements directly in aqueous biological systems. As an alternative to LSC, we developed polystyrene-core, silica-shell nanoparticle scintillators (referred to as nanoSCINT) for quantification of low-energy β -particle emitting radionuclides directly in aqueous solutions. The polystyrene acts as an absorber for energy from emitted β -particles, and can be loaded with a range of hydrophobic scintillant fluorophores, leading to photon emission at visible wavelengths. The silica shell serves as a hydrophilic shield for the polystyrene core, enabling dispersion in aqueous media and providing better compatibility with water-soluble analytes. While polymer and inorganic scintillating microparticles are commercially available, their large size and/or high density complicates effective dispersion throughout the sample volume. In this work, nanoSCINT nanoparticles were prepared and characterized. nanoSCINT responds to ^3H , ^{35}S , and ^{33}P directly in aqueous solutions, does not exhibit a change in scintillation response between pH 3.0 and 9.5 or with 100 mM NaCl, and can be recovered and reused for activity measurements in bulk aqueous samples, demonstrating the potential for reduced production of LSC waste and reduced total waste volume during radionuclide quantification. The limits of detection for 1 mg/mL nanoSCINT were 130 nCi/mL for ^3H , 8 nCi/mL for ^{35}S , and $< 1 \text{ nCi/mL}$ for ^{33}P .

Graphical Abstract

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Supporting Information Available

Additional data supporting characterization and optimization of nanoSCINT. Supporting information is available free of charge on the ACS publications website at DOI: **Insert upon publication**

Keywords

scintillation; core-shell nanoparticle; β -emission; radioisotope; composite nanoparticle

INTRODUCTION

Radionuclides, specifically ^3H , ^{33}P , and ^{35}S (all β -particle emitters), are commonly used as labels in biological assays due to the distribution of these atoms in biological systems.^{1–8} Unlike fluorophores or fluorescent protein tags, radionuclides do not significantly increase the size or mass of the labeled component, and therefore have minimal effects on binding, conformational changes, diffusion and active transport, etc. In addition, lower backgrounds are typically obtained for radioassays compared to fluorescence assays, where background arises from the inherent fluorescence of many species within the sample.^{9–11} Radioassays have been used to quantify antigens at sub-pM concentrations, drugs such as buprenorphine at sub-nM concentrations,^{1, 8} and enzyme activity.^{4–5}

β -particle emission from radiolabeled analytes is commonly quantified using scintillation counting techniques. Scintillation occurs when the energy of a particle (α , β or photon) emitted during radioactive decay is absorbed by excitable species within the medium to an excited electronic state. Relaxation to the ground state results in emission of a photon or the transfer of energy to another molecule, which may subsequently undergo photoemission.¹² Aqueous samples are usually dispersed in scintillation cocktails (LSCs), which are comprised of mixtures of aromatic, absorbing hydrocarbons (e.g., benzene, toluene, xylene, diisopropylnaphthalene, alkylbenzenes), surfactants, and scintillant fluorophores.^{12–13} While useful, the high solvent composition precludes real time analysis directly in aqueous solutions, such as those required for monitoring biological function, and limits the amount of aqueous sample that can be added to LSC without phase segregation. Additionally, measurements in LSC generate large volumes of radioactive mixed waste that must be collected and disposed according to state and federal regulations. The toxicity and volatility of the primary solvent components of many LSC formulations complicate their transport, storage, and disposal.

Solid scintillant materials prepared from dye-doped polymers or scintillating inorganic glasses provide an alternative to detect β -emission and have the advantage of functioning in aqueous environments.^{14–15} Doped glass matrices can be ground into microparticles with irregular shapes and relatively large particle sizes compared to synthetically produced particles. Polymer matrices that incorporate scintillant fluorophores facilitate the transfer of energy from the β -emission to visible photons.^{16–18} Polymer materials can be formed into

microparticles or molded into various sample geometries, including 96-well plates, that enable high throughput detection. Due to the minimized organic solvent, solid materials generate much less organic waste. The primary drawback of solid scintillation counting is the reduced scintillation efficiency that arises from the increased separation distance between scintillating particles and individual β -emission events compared to dyes dispersed in solvent, leading to poor photon conversion. For example, β -particles arising from ^3H emission exhibit a mean penetration depth of ca. 500 nm in water.¹⁹ The increased separation distance results from settling of the high-density glass microparticles and/or the low effective concentrations of microparticles that can be used due to particle sizes. Additionally, the relatively large size leads to poor dye accessibility for β -energy conversion. Thus, alternative approaches for measuring low-energy radionuclide activity are increasingly attractive.

In this work, we describe a polystyrene-core silica-shell solid nanoparticle scintillator (nanoSCINT) for use in low-energy radionuclide quantification. The scintillant fluorophore-doped, polymer matrix facilitates energy transfer and photon conversion whereas the addition of a silica shell increases hydrophilicity and enables dispersion of polyvinyltoluene or polystyrene nanoparticles directly in aqueous samples.^{20–21}

EXPERIMENTAL

Materials

Styrene, alumina, p-terphenyl (pTP), and 1,4-bis(4-methyl-5-phenyl-2-oxazolyl)benzene (dmPOPOP) were purchased from Acros Organics (NJ). Tetraethylorthosilicate (TEOS), and 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AIBA), 2,2'-azobis(2-methylpropionitrile) (AIBN), Triton X-100, cyclohexane, sodium citrate (tribasic) hydrate, sodium tetraborate hydrate, and 2-(N-morpholino)ethanesulfonic acid hydrate (MES) were obtained from Sigma Aldrich (St. Louis, MO). Sodium chloride, sodium phosphate hydrate (monobasic), isopropyl alcohol (IPA), chloroform, tetrahydrofuran (THF), and aqueous ammonium hydroxide (28%) were obtained from EMD Millipore (Billerica, MA). Hexyl alcohol and divinylbenzene (DVB) were purchased from Alfa Aesar (Haverhill, MA). Ethyl alcohol was purchased from Decon Laboratories (King of Prussia, PA). BioCount liquid scintillation cocktail was acquired from Research Products International (Mt. Prospect, IL). ^3H -labeled sodium acetate was purchased from Perkin Elmer (Waltham, MA). All chemicals except styrene and DVB were used as received. Inhibitor was removed from styrene and DVB by passing the monomers through 0.5 cm diameter by 3 cm long alumina columns immediately prior to use.

Preparation of nanoSCINT Nanoparticles

Styrene (3 g, inhibitor-free) was added to 100 mL degassed H_2O in an Ar-flushed 500 mL round-bottomed flask heated to 70 °C in an oil bath. Polymerization was initiated by adding 10 mg of AIBA dissolved in approximately 200 μL of H_2O to the reaction flask. For experiments comparing polystyrene core nanoparticles polymerized using AIBA to core nanoparticles polymerized using AIBN, core nanoparticles were synthesized following the same method except that AIBA was replaced with the same mole amount of AIBN dissolved

in 1 mL ethyl alcohol. For comparison of crosslinking ratio, inhibitor-free DVB was combined with styrene at 1 or 2 mol %, as indicated. The H₂O/styrene mixture was stirred rapidly for at least 6 h. Excess styrene was removed from the nanoparticle solution under reduced pressure using a rotary evaporator. The total volume of the nanoparticle solution was reduced to 100 mL, and a small aliquot (1–2 mL) of the solution was removed and lyophilized to determine the weight per volume of nanoparticles.

Polystyrene nanoparticles were doped with scintillant fluorophores by dissolving 53 mg (135 μ moles) of dmPOPOP and 262 mg (1.14 mmoles) of pTP in 20 mL of 1:9 isopropanol chloroform (v:v), except for comparison to THF, in which case 20 mL THF was used. Scintillant fluorophores dissolved in solvent were added directly to the aqueous polystyrene nanoparticle solution in a 500 mL round-bottomed flask. The nanoparticle solution was sonicated using a bath sonicator for several minutes to disperse organic solvent droplets throughout the H₂O and the solution was stirred rapidly for at least 1 h. Organic solvents were then removed under reduced pressure using a rotary evaporator. For experiments in which the ratio of pTP to dmPOPOP was optimized, the amounts of pTP and dmPOPOP were adjusted to give the indicated concentrations, but the process for introducing the scintillant fluorophores into the polystyrene nanoparticles was the same. Once solvent was removed, the scintillant fluorophore-doped polystyrene nanoparticle solution was stored at room temperature (24 ± 1 °C) until use. The diameter of the doped polystyrene cores was measured by dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS) and found to be 190 ± 2.8 nm with a polydispersity index (PDI) of 0.064, where PDI is defined as two times the third order cumulant (c) divided by the second order cumulant (b) squared, of the polynomial fit to the correlation function by cumulants analysis.

Silica shells were added to scintillant fluorophore-doped polystyrene nanoparticles by dispersing 2 mL polystyrene nanoparticle stock solution (approximately 56 mg of nanoparticles) in 200 mL isopropanol with 38 mL H₂O and 5 mL NH₄OH. The dispersion was stirred briskly for several minutes while 2 mL TEOS was added dropwise. Stirring was continued for 1 h before nanoSCINT nanoparticles were collected by centrifugation and rinsed several times with H₂O. nanoSCINT particle diameter was measured to be $270 \text{ nm} \pm 0.85 \text{ nm}$, with a PDI of 0.049.

For surface charge comparisons, silica nanoparticles without polystyrene cores were prepared by dispersing 1.8 mL Triton X-100 in a mixture of 8.0 mL cyclohexane and 1.8 mL hexyl alcohol in a 50 mL round bottom flask. The mixture was stirred briskly with a stir bar for several minutes before 550 μ L H₂O, 60 μ L NH₄OH and 100 μ L TEOS were added. Stirring was continued for approximately 12 h. Silica nanoparticles were collected by adding several mL of acetone to flocculate nanoparticles at the bottom of the flask. Nanoparticles were rinsed three times with 1.5 mL aliquots of ethyl alcohol and followed by three times with 1.5 mL aliquots of H₂O. The silica nanoparticles were measured to be $170 \text{ nm} \pm 0.55 \text{ nm}$ in diameter, with a PDI of 0.24.

Transmission Electron Microscopy

Transmission electron micrographs of polystyrene and nanoSCINT nanoparticles were obtained by drying a 10 μ L aliquot of nanoparticle solutions on carbon films on copper grids

(Ted Pella, Inc, Redding CA). Samples were observed using a Tecnai G2 Spirit transmission electron microscope at 100 kV accelerating voltage (FEI Company, Hillsboro OR).

Zeta Potential Measurements

Zeta potential of nanoparticles with differing compositions was measured using a Zetasizer Nano (Malvern Instruments, Ltd., Malvern, UK). Polystyrene, silica, and nanoSCINT nanoparticles were dispersed in 100 mM NaCl at varying pH (from 3.0 to 10.0) immediately prior to measurement. Zeta potential was calculated using the Smoluchowski approximation as the solution for the Henry equation for all samples.²²

Scintillation Response Measurements and nanoSCINT Particle Recovery

The scintillation response was evaluated as a function of primary to secondary scintillant fluorophore ratio using polystyrene core nanoparticles doped with 1:0, 1:1, 1:10, 1:100, 1:1000, 1000:1, 100:1, 10:1, and 0:1 molar ratios of pTP:dmPOPOP. Polystyrene core nanoparticles suspended in H₂O were combined with ³H-labeled sodium acetate (150 mCi/mmol) to make 0, 0.25, and 0.5 μ Ci/mL samples. Scintillation response, in counts per minute, was measured for each sample after each addition of ³H-labeled sodium acetate using a Beckman LS 6000IC liquid scintillation counter, as well as for polystyrene core nanoparticles lacking primary or secondary scintillant fluorophores.

Scintillation response as a function of scintillant fluorophore doping was evaluated by dispersing equal amounts of nanoSCINT (based on the mass of polystyrene cores used during shell synthesis, since not all silica nanoparticles may contain scintillating polystyrene cores) prepared using different scintillant fluorophore doping procedures. Fluorophore doping was determined by the fluorescence emission intensity ($\lambda_{\text{ex}} = 270$ nm; $\lambda_{\text{em}} = 300$ to 550 nm) using a Quantamaster fluorimeter (Photon Technology International, Birmingham, NJ). The comparative scintillation response was then measured by adding ³H-labeled sodium acetate (150 mCi/mmol) to equal amounts of nanoSCINT dispersed in H₂O.

To evaluate the feasibility of recovering and reusing nanoSCINT, the scintillation response of recovered nanoSCINT was determined by combining fixed amounts of nanoSCINT nanoparticles in H₂O with ³H-labeled sodium acetate. A 2 mL nanoSCINT particle stock solution was added to each of three 7 mL polyethylene scintillation vials. ³H-labeled sodium acetate (150 mCi/mmol) was sequentially added to the nanoSCINT samples, as well as to each of three vials containing 2 mL each of H₂O only, and three vials containing 2 mL each of LSC. The scintillation response, in counts per minute (CPM), was measured for each sample after every addition of ³H-labeled sodium acetate. After the final addition of ³H-labeled sodium acetate, nanoSCINT nanoparticles were recovered from each sample by centrifugation at 16,000 g for 5 min. Each recovered nanoSCINT sample was rinsed once by re-suspending the nanoparticles in 5 mL fresh H₂O and then collected by centrifugation. nanoSCINT nanoparticles were then resuspended in 2 mL of H₂O and treated with increasing activities of ³H-labeled sodium acetate to measure the response.

The effects of pH and ionic strength on nanoSCINT response were explored by dispersing fixed amounts of nanoSCINT nanoparticles in 10 mM buffer (sodium citrate at pH 3.0, MES at pH 5.5, sodium phosphate at pH 7.0, or sodium tetraborate at pH 9.5) and measuring

scintillation before and after the addition of ^3H -labeled sodium acetate. The ionic strength was increased by adjusting the NaCl concentration to 100 mM and scintillation response measured.

Data analysis and presentation

All results are presented as the mean $\pm$ standard deviation of a minimum of three different replicates. Error bars in all plots represent the standard deviation of the measurements.

RESULTS AND DISCUSSION

Traditional LSC functions via energy absorption by an aromatic solvent followed by energy transfer to and photon emission from scintillant fluorophores. Additives, including surfactants, are used to facilitate the dispersion of aqueous samples into the organic solvent, which leads to a loss of biological activity upon dispersion. The scintillant fluorophores are included to increase the emission intensity and to shift photon emission to wavelengths more readily detected by photomultiplier tubes and CCDs. Solid polymer scintillation particles provide an alternative to LSC where the polymer (e.g. polystyrene or polyvinyltoluene) replaces the organic solvent base as the primary energy absorber from β -particle emission. Although the π -orbital electrons of polystyrene itself are readily excited by β -particle emission, the radiative quantum yield of polystyrene is low (ca. 7%),¹⁷ necessitating scintillant fluorophores (in this work pTP and dmPOPOP) to increase emission intensity and shift the emission to readily detectable spectral windows.^{16–18} The large size and poor solubility of polymer microparticles limits the utility and sensitivity of this approach in the current format.

To overcome these limitations, we utilized the polymer scintillant approach to prepare composite, hydrophobic core- hydrophilic shell scintillating nanoparticles, referred to as nanoSCINT, that function directly in an aqueous environment. The shift to nanoparticles significantly increases the surface area per unit volume of the scintillant particles in solution compared to commercially available 5–10 μm particles. The core-shell geometry increases dispersibility and minimizes aggregation by optimizing the interfacial properties between the solution and particle surface. The shell also provides a platform for modification in future sensor applications. nanoSCINT was initially prepared by synthesizing polystyrene nanoparticles doped with scintillant fluorophores and subsequently adding a thin silica shell to the scintillating polystyrene nanoparticles creating the core-shell morphology as illustrated in Scheme 1.

To prepare functional nanoSCINT, we initially focused on the development of scintillating polystyrene cores that enable high β -emission to photon conversion and that can subsequently serve as the core in the nanoSCINT platform. Scintillant fluorophore-doped polystyrene core nanoparticles were fabricated via surfactant-free emulsion polymerization using the cationic initiator AIBA, as illustrated in Scheme 1. The core nanoparticles were then doped with primary and/or secondary scintillant fluorophores using a particle swelling/deswelling procedure after initial attempts at *in situ* doping were ineffective due to dye solubility issues. In this approach, the nanoparticles are first swelled using solvent containing dissolved scintillant fluorophores, and then deswelled by slowly removing the

solvent, leaving the scintillant fluorophores entrapped within the polymer matrix.^{23–24} For this proof-of-concept work, initial efforts to maximize the scintillation response focused on the frequently used scintillant fluorophores pTP and dmPOPOP. Energy absorbed by polystyrene is transferred to the primary scintillant fluorophore, pTP. Further energy transfer occurs from pTP to the secondary scintillant fluorophore, dmPOPOP, which emits photons at blue wavelengths (maximum intensity between 400 and 450 nm) that is more readily detected by scintillation counters.

Core Optimization

After initially determining that dye could be encapsulated within the polystyrene cores using this approach, we investigated the effects of dye composition. The ratio of primary to secondary scintillant fluorophore plays an important role in the overall scintillation efficiency of polymer scintillators. To optimize the scintillation response, we entrapped different ratios of pTP and dmPOPOP in the polystyrene core nanoparticles and measured the scintillation response with increasing ³H. pTP:dmPOPOP ratios ranging from 1000:1 to 1:1000 were evaluated (Supporting Information, Table S1). pTP:dmPOPOP ratios of 1:1 and 10:1 yielded the highest sensitivity for ³H response and were therefore used in subsequent experiments. It should be noted that the net scintillation response for nanoSCINT is governed by a complex interplay of primary scintillant fluorophore concentration, scintillant fluorophore solubility and the partition coefficient into polystyrene, as well as total scintillant fluorophore concentration, presenting further avenues for future optimization.

Once the optimal ratio of scintillant fluorophores on radioisotope response was identified, we investigated the effects of solvent composition and polystyrene composition on dye loading. To best encapsulate scintillant dyes, maximum swelling is necessary; conversely, excessive swelling may lead to solubilization of the polystyrene cores. Polystyrene core nanoparticles prepared without crosslinking agents are soluble in many solvents. Crosslinking the polystyrene with DVB may prevent dissolution, but also restrict the entrapment of scintillant fluorophores in the polymer matrix.

Polystyrene core nanoparticles prepared with 0, 1 or 2 mol % DVB crosslinker were swollen by the addition of either THF or a 9:1 mixture of CHCl₃:IPA (Supporting Information, Figure S1). Upon characterization of the fluorescence intensity, an indicator of scintillant fluorophore concentration, or ³H response, we found that polystyrene cores with 2% DVB swollen in CHCl₃:IPA demonstrate the highest scintillation response, whereas the polystyrene cores lacking DVB and swollen in CHCl₃:IPA contain the greatest concentration of scintillant fluorophores. The higher fluorescence intensity of the 0% DVB sample may result from greater swelling and unhindered absorption of scintillant fluorophores into the polymer, but the enhanced scintillation response of the 2% DVB sample may enhance energy transfer by increasing the rigidity of the polymer. Interestingly these results align well with prior investigations of organic scintillators dispersed in polystyrene sheets where enhanced energy transfer was observed in more rigid polymers and differences in emission output were observed for high energy (x-ray, γ or β -emission) excitation compared to UV-excitation.^{25–27} Since an enhanced scintillation response is not observed with increasing

DVB percent for all samples, there is likely a balance between the degree of swelling (depending on the solvent) and the effect of DVB on energy transfer.

After the addition of the silica shells, scintillant fluorophore doped polystyrene cores exhibited a decrease in both scintillation response and fluorescence, and this effect was greater for more highly crosslinked cores. The reduction in dye loading and scintillation response may result from dye extraction during the shell synthesis, which utilizes a large volume of IPA. Although the solubility parameters of pTP ($17.3 \text{ MPa}^{1/2}$) more closely match that of polystyrene ($18.7 \text{ MPa}^{1/2}$) compared to IPA ($24.4 \text{ MPa}^{1/2}$),^{28–29} the large volume difference between polystyrene and IPA likely leads to significant dye loss (Supporting Information, Figures S1 and S2). Interestingly, after the addition of silica shells to the scintillant fluorophore-doped polystyrene cores (discussed in more detail below), the sample with 0% DVB swollen in CHCl_3 :IPA shows both the highest fluorescence intensity and the greatest scintillation response (Supporting Information, Figure S2), most likely because of the higher initial dye loading into polystyrene cores with 0% DVB which may offset dye loss during silica shell synthesis. For all further studies, polystyrene cores lacking DVB were utilized.

Once optimal compositions for polystyrene cores were identified, the performance characteristics for detection of low energy radioisotope detection were evaluated prior to nanoSCINT synthesis. Scintillation response to ^3H was measured as a function of nanoparticle concentration and of increasing ^3H activity (Figure 1). Increased polystyrene core concentrations yielded an increase in photoemission response to a fixed ^3H activity from the core nanoparticles as indicated by the scintillation response. Since ^3H β -particles have short penetration depths (ca. 500 nm on average) in aqueous solution, the increase in response corresponds directly to the anticipated decrease in interparticle spacing at higher particle concentrations (Figure 1A). Once the particle concentration dependence was established, we investigated the response to increasing ^3H activity using a fixed concentration of polystyrene cores. Under these conditions, increases in ^3H activity yielded a linear scintillation response (Figure 1B). In both cases, when polystyrene core nanoparticles that lack scintillant fluorophores were used, no response was observed, demonstrating that the polystyrene matrix itself does not contribute to signal background at the nanoparticle concentrations and activities used here.

Synthesis and characterization of nanoSCINT

It has been suggested that surface charge greatly affects the formation of silica shells on polymer nanoparticles due to electrostatic interactions between the polymer and the silica oligomers.^{30–33} Initial attempts to prepare nanoSCINT in which styrene was polymerized using the neutral initiator AIBN formed mixtures of polystyrene nanoparticles and silica nanoparticles during the silica addition step. One possible explanation for this observation is that the neutral or negative polymer formed from styrene polymerized with AIBN has few nucleation sites that lead to enhanced interaction with the silica precursors.³² In such a case, silica may be more likely to nucleate and grow into silica nanoparticles in the reaction mixture rather than form silica shells on polystyrene cores.³³ To overcome this limitation, polystyrene core nanoparticles were synthesized using a cationic initiator, which imparts an

overall positive charge to the nanoparticles.^{31–32} Zeta potential measurements for polystyrene core nanoparticles (Supporting Information, Figure S3) show that core nanoparticles polymerized using AIBN exhibit a negatively (–20 to –30 mV) charged surface, while core nanoparticles polymerized with AIBA have a positively (20 to 30 mV) charged surface except at high salt concentration (100 mM NaCl), where the zeta potential is near 0 mV. Cationic nanoparticles, such as those composed of styrene polymerized using AIBA, may accumulate anionic silica oligomers and polymeric chains, which develop into silica shells as the hydrolysis and condensation reactions proceed. Thus, AIBA was used to prepare polystyrene cores used for further nanoSCINT preparation.

To prepare nanoSCINT, a dispersion of scintillant fluorophore-doped polystyrene cores was prepared in mixture of IPA, H₂O and NH₄OH. TEOS was added to the solution and allowed to react for 1h (Scheme 1). TEM images were obtained for polystyrene cores and nanoSCINT nanoparticles. Observation of the morphology and size of nanoSCINT compared to the polystyrene core revealed a more electron dense shell surrounding the polymer core (Figure 2), consistent with prior reports of polystyrene:silica core shell composites.^{32, 34} In the images, the denser silica shells appear darker than the less dense polystyrene cores (Figure 2B), while shell-free polystyrene cores appear uniformly dark upon the carbon film background. Partial dissolution of the polystyrene cores from nanoSCINT in toluene showed a slight enhancement in the contrast between the core:shell interface (Supporting Information, Figure S4). The shell thickness was approximately 30 nm (ranging from 25–35 nm) and appeared uniformly distributed around the nanoparticles. Previous research in this field has demonstrated that silica shell thickness can be coarsely adjusted by altering a number of variables including, base concentration, silane concentration, and time, although precise control is not possible.^{33, 35–36} Results from our initial experiments indicate that base (NH₄OH) concentration was the most important factor in controlling silica shell formation, as it was observed that a higher concentration of NH₄OH in the reaction mixture leads to thicker shells (greater than 100 nm).

The surface charge characteristics of nanoSCINT were examined by comparing the zeta potentials of nanoSCINT nanoparticles to the zeta potentials of silica and polystyrene core nanoparticles (Figure 2C). The zeta potentials of silica nanoparticles become increasingly negative with increasing pH due to deprotonation of surface silanols; at pH 3, the zeta potential is approximately neutral (+1 mV). In contrast, at pH 10 the zeta potential decreases to approximately –30 mV upon complete deprotonation of the surface silanols. Interestingly, the zeta potentials of polystyrene core nanoparticles are also negative; the zeta potential at pH 3 is approximately –5.0 mV, and approaches –18 mV at pH 10. As described above, the cationic initiator AIBA was used for the fabrication of polystyrene core nanoparticles to make the overall surface charge of the nanoparticles positive. The polystyrene surfaces may therefore be decorated with amidine moieties originating from AIBA that can undergo hydrolysis over time, and with increasing pH,^{37–38} to form amide groups, leading to a negative zeta potential of greater magnitude. However, it should be noted that polystyrene cores are stored in water rather than in high pH solutions, and that the silica shell addition process is performed in low ionic strength solutions, thus the surface charge of polystyrene nanoparticles prepared using AIBA is more likely to remain either neutral or positive under the conditions of silica shell synthesis as shown in the Supporting Information Figure S3.

nanoSCINT nanoparticles exhibit a very similar trend to those observed for silica nanoparticles with the zeta potential generally matching that of silica throughout the pH range, further supporting the presence of a silica shell.

Importantly, these data support the utility of nanoSCINT over a range of salt concentration and pH values, critical parameters for the dispersion of nanoSCINT in aqueous solutions. Although silica is considered lyophilic, electrostatic repulsions between nanoparticles (directly related to the fraction of deprotonated silanols and ions present at the particle surface) at least partially dictate whether or not nanoparticles resist flocculation/aggregation.^{39–42} When the surface charge of nanoSCINT is close to zero, e.g. at pH 4 (-5 mV), it is possible that nanoSCINT may aggregate, reducing scintillation response by reducing contact with solvated ^3H -labeled species. To evaluate this possibility, scintillation response of nanoSCINT to ^3H was measured from pH 3.0–10.0 in the presence and absence of 100 mM NaCl (Figure 2D). These data demonstrate clearly the ability to detect ^3H β -emission using nanoSCINT. Further, no statistically significant differences were observed in the scintillation response between conditions, supporting the utility of nanoSCINT under these diverse solution conditions.

Similar to the characterization of scintillant fluorophore-doped polystyrene cores, we sought to determine the response of nanoSCINT to increasing ^3H activity. Figure 3 shows the response of nanoSCINT nanoparticles and corresponding polystyrene cores to increasing ^3H activity. A linear response was observed, analogous to that for polystyrene cores (Figure 3A). A < 2 -fold lower net response was obtained for nanoSCINT compared to polystyrene cores. The most likely explanation for this observation is the removal of adsorbed scintillant fluorophore molecules or loss of scintillant fluorophores from the polystyrene during the silica shell addition to reduce the total scintillant fluorophore present per unit mass of nanoSCINT nanoparticles.

Additionally, we compared nanoSCINT to LSC. Scintillation experiments reveal that polystyrene core nanoparticles and nanoSCINT demonstrate a 500–1000 fold lower light emission at 2 μCi ^3H activity compared LSC (Figure 3B). This is not unexpected due to the markedly higher scintillant concentration in the LSC. Importantly, even at lower net scintillation response compared to LSC, nanoSCINT can be used to detect and quantify low-energy β -emitters such as ^3H in aqueous solution at nCi activity levels. While this sensitivity is less than LSC, nCi-mCi measurements are normally used for biological radioisotope experiments, supporting the utility of nanoSCINT.

To investigate the potential of nanoSCINT for reuse, and thereby the reduction of LSC-related waste, we evaluated the response of nanoSCINT for multiple measurements. Figure 3A shows the response nanoSCINT nanoparticles used to measure ^3H activity and then the response of the same nanoSCINT nanoparticles after recovery via centrifugation and a single wash with H_2O . The response of the recovered nanoSCINT nanoparticles was ca. 1.5x higher at the highest ^3H activity used compared to the initial nanoSCINT response. This difference in response may be acceptable for measurements at low activity, though the ultimate application will dictate whether this variability is an acceptable tradeoff for the

enhanced capabilities in completely aqueous samples and waste reduction enabled by nanoSCINT.

Finally, the long-term goals for nanoSCINT involve detection of β -emission from multiple biologically relevant isotopes. Thus, we evaluated the response of nanoSCINT nanoparticles to higher energy β -particles from commonly used ^{35}S and ^{33}P ($E_{\text{max}}=167$ keV and 249 keV, respectively) compared to ^3H ($E_{\text{max}} = 18.6$ keV).¹² The corresponding scintillation response was linear for each isotope measured and the trend observed was $^{33}\text{P}>^{35}\text{S}>^3\text{H}$ with respect to scintillation response (Figure 4). The limits of detection for 1 mg/mL nanoSCINT were calculated to be approximately 130 nCi/mL for ^3H , 8 nCi/mL for ^{35}S , and < 1 nCi/mL for ^{33}P . Most importantly, these data demonstrate the utility of nanoSCINT for measuring multiple radioisotopes that are used in wide ranging biological studies.

Conclusions

nanoSCINT nanoparticles, which are composed of scintillant fluorophore-doped polystyrene core nanoparticles surrounded by a silica shell were developed and can be used to quantify low-energy radionuclides such as ^3H , ^{35}S , and ^{33}P in aqueous samples. Although not as efficient as LSCs, due in part to sample geometry, nanoSCINT is recoverable and re-useable, which may reduce waste and allow for sample recovery. Unlike traditional LSC formulations, nanoSCINT nanoparticles are made from non-toxic and non-volatile components^{43–47} and can be used directly in aqueous environments without the aid of surfactants and over a broad range of pH and ionic strength, potentially enabling key time-resolved studies of chemical and biological activity in aqueous samples using radioisotopes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health via the National Institute of Biomedical Imaging and Bioengineering under grant number R21EB019133 and the National Institute of General Medical Sciences under grant number 1R01GM116946. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Figure 1.

Scintillation response of scintillant polystyrene core nanoparticles lacking (squares) and containing (circles) scintillant fluorophores. A) Effect of particle concentration. Polystyrene core concentrations were increased in the presence of fixed ^3H activity (300 nCi). B) Effect of ^3H activity. ^3H activity was increased in the presence of a fixed polystyrene core concentration (3.2 mg/mL).

Figure 2.

Characterization of nanoSCINT nanoparticles. TEM images of A) polystyrene core nanoparticles and B) core-shell nanoSCINT nanoparticles; C) Zeta potential measurements of nanoSCINT nanoparticles (circles), polystyrene core nanoparticles without silica shells (squares), and silica nanoparticles without polystyrene cores (triangles). D) Scintillation response of nanoSCINT nanoparticles in the presence (triangles and squares) and absence (circles) of $1 \mu\text{Ci}$ ^3H -labeled sodium acetate as a function of pH at high (squares) and low (triangles) salt concentration.

Figure 3.

Scintillation response of nanoSCINT to increasing ^3H activity. A) Scintillation as a function of ^3H -labeled sodium acetate for nanoSCINT (square), scintillant polystyrene cores (cross), water lacking scintillant fluorophores (diamonds) and recovered nanoSCINT (triangles). B) Scintillation response for LSC (circles) and nanoSCINT (squares). The nanoparticle concentrations were 2.8 mg/mL for each nanoparticle enabled measurement. Scintillation response was normalized to the fluorescence of the nanoSCINT sample or of liquid scintillation cocktail at $\lambda_{\text{ex}} = 270 \text{ nm}$ and $\lambda_{\text{em}} = 430 \text{ nm}$.

Figure 4.

Scintillation response of nanoSCINT to key biologically relevant radioisotopes. ^3H (blue circles), ^{35}S (red squares), and ^{33}P (green triangles) activity was increased and scintillation response measured. The y-axis provides a relative number for the response for each isotope. For the absolute value for ^3H response, the axis ranges from (0–400), for ^{35}S from (0–4,000) and for ^{33}P from (0–40,000).

Scheme 1.

Illustration of the polystyrene-core silica-shell nanoSCINT preparation process. Core polystyrene nanoparticles are first synthesized using a surfactant-free polymerization process. Silica shells are added to the cores in a subsequent condensation reaction.