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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are considered as priority pollutants by the EPA since they are widespread, serious risks for ecosystems and human health due to their carcinogenic and mutagenic effects. PAHs have low water solubility, and are strongly adsorbed onto environmental surfaces, accumulating in soils and sediments. Elevated levels of PAHs have been detected in urban (sub)surface soils throughout the US, in concentrations often several orders of magnitude greater than established EPA screening levels. We have previously shown that SiO₂ nanoparticle-supported lipid bilayers (NP-SLB) formed by using inexpensive, environmentally-friendly geomaterials, such as SiO₂ nanoparticles, as well as a natural organic matter component, lipids, can remove PAHs. Here, we aim to examine the stability and feasibility of the remediation method under various environmental conditions by tracing their migration in different environments. First, we successfully synthesized the composite material with approximately 200-nm SiO₂ nanoparticles, fluorescently-labelled with Alexa Fluor 350 NHS Ester dye and lipids tagged with a DiA styryl dye, which allowed us to trace them separately for testing the stability and integrity of the composite material. Further, we fabricated optically transparent plates with 2-D channels that are composed of SiO₂ and SiO₂ surfaces functionalized with humic acid and/or clay minerals to closely simulate heterogeneity and complexity in soil matrices. Dynamic light scattering, transmission electron microscopy, spectrofluorometer, and nanodifferential scanning calorimetry were employed for the detailed material characterization and measurements. Results of this study indicated that SiO₂ NP-SLB is a robust and stable composite material, desirable for in-situ remediation of PAHs in soils. Further, micro-model studies clearly presented advantages over batch sorption/desorption experiments or soil column experiments by visualizing both NPs and lipids differently in the presence of various environmental surfaces. Such successful delivery of the intact and stable composite material to the target contaminated sites expects to increase the efficiency of NP-SLB system for the PAHs removal in soils.

Objectives

Here we report on a micro-model system with 50-100 mm (width), 100 mm (depth) channels with large aspect ratios that have either linear, once bifurcated or tortuous path channels. This micro-model permits the use of very small quantities of NPs, the ability to change flow rates easily, and to observe deposition as a function of channel wall composition and topology. By varying the composition of the channel walls, heterogeneous surfaces, more similar to those found naturally, can be prepared, and heterogeneous NP-surface interactions, believed to be important in determining transport behavior of colloidal particles¹, can be investigated. The micro-models are transparent and can be fabricated with any network structure/topology, so they can better simulate the complexities of natural porous media, and identify the hydrochemical and hydrodynamic factors that affect transport and deposition processes of NPs at the pore level. Information obtained from the micro-model studies can provide more detailed understanding about the mobility of natural and manufactured NPs in the environment than that provided by flow through columns since local fluid

and NP dynamics can be directly monitored. In column experiments, preferential flow pathways are obscured and attachment efficiencies are measured on length scales corresponding to column (not pore) dimensions.

Materials and Methods

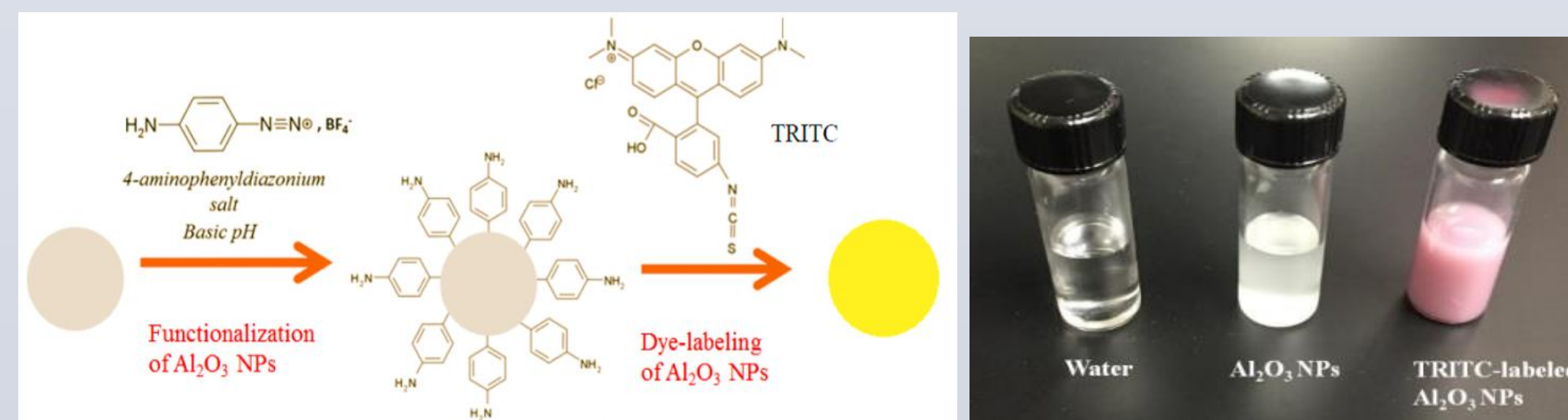
Chemicals: Absolute ethanol (EtOH), HPLC grade water, tetraethylorthosilicate (TEOS) and (3-aminopropyl) triethoxysilane (APTES) (Thermo-Fisher Scientific) were used for the synthesis. TEOS and APTES were freshly distilled before each synthesis. Ammonium hydroxide, which contained 14.0 M NH₃, was used as received. The dyes fluorescein isothiocyanate (FITC, isomer I, Sigma-Aldrich) and Alexa Fluor 350 NHS Ester dye (Life Technologies), were used as received. Centrifugations were done on add. Dynamic light scattering and zeta potential measurements were obtained on Malvern Zetasizer Nano-Analyzer, and TEM images were obtained using JEOL JEM-1400 transmission electron microscope. UV-Vis and fluorescence data were acquired on micro-channels were 100 mm x 100 mm and 12.5 mm long.

Formation of fluorescent small unilamellar vesicles (SUVs) and supported lipid bilayers (SLBs)

Small unilamellar vesicles (SUVs), size 100 nm diameter, in which 3% of the fluorescent lipid 4-Di-16-ASP (4-(4-(dihexadecylamino)styryl)-N-methylpyridinium iodide; C₄₆H₇₉N₂, 787.048 g/mol (DiA-16) (Invitrogen, Thermo-Fisher Scientific, NY) (**Figure 1**) was incorporated, were prepared as described elsewhere². Briefly, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and DiA-16/1 were codissolved in chloroform and the chloroform evaporated, followed by drying overnight under vacuum. The dry lipids were hydrated in water or salt solutions at 40 °C, freeze/thawed 5x to form multilamellar vesicles (MLVs) and extruded (40x) through 100 nm polycarbonate filters in an Avanti Polar Lipids extruder at 40 °C to form small unilamellar vesicles (SUVs). The SUVs had z-average diameters (D_z) of 100 nm, and a slightly positive zeta potential of +14mV. The UV-Vis absorption maximum of the SUVs was 460 nm and their emission maximum was 580 nm. These same vesicles were used to form supported lipid bilayers (SLBs) on nominal 100 nm diameter silica nanoparticles (Nissan Chemical America Corp. Houston, TX), as described elsewhere³, by incubation of the SUVs and SiO₂ NPs for 1 h at 40 °C. Samples were prepared so that the nominal surface areas (SAs) of the 100 nm SiO₂ and the SUVs were the same, i.e. where SA_{SUV}/SA_{SiO₂} = 1, or where 2x this amount of lipid was used, i.e. SA_{SUV}/SA_{SiO₂} = 2/ In this case, there were approximately equal populations of SUVs and SLBs. Suspensions were prepared either in distilled water, 5 mM NaCl, 10 mM NaCl or 100 mM NaCl.

Synthesis of fluorescent nanoparticles

Fluorescently labelled SiO₂, in a range of sizes (**Figure 2** shows ~ 200 nm NPs) and dyes (e.g. fluorescein^{4,5} (FITC), tetramethylrhodamine (TRITC), Alexa (all Thermo-Fisher Sci.) were prepared using a modified Stöber process for SiO₂, where the dye was incorporated within the bulk or the exterior of the NPs⁴⁻⁷. Fluorescently modified SiO₂ NPs were synthesized with overlayers of SiO₂⁸⁻⁹ in order to shield the fluorescent dye and reintroduce SiOH groups necessary for SLB formation (otherwise there are residual amines). The dye FITC was attached to APTES by an addition reaction between the amine group and the thioisocyanate group⁵ to form the N-1-(3-trimethoxysilylpropyl)-N-fluoresceyl thiourea derivative. FITC and excess APTES were slowly stirred for at least 12 h in anhydrous ethanol, to prevent self-condensation of the APTES. Typically 1-2 mL of EtOH was used for 0.1g APTES. This reaction product was added to ammonia in distilled EtOH at 25 °C, followed by addition of distilled 0.17M TEOS, and this solution was stirred for at least 6 h. The SiO₂/APTES-dye particles were centrifuges/rinsed in distilled water, and used this way or subsequently further coated with another layer of SiO₂. The addition of a final SiO₂ layer resulted in NPs that were fluorescent but which had similar surface properties/potentials as homogeneous SiO₂ spheres, so that supported lipid bilayers could be easily formed. A general method for surface functionalization of metal oxide NPs using aryldialonium salts¹⁰ was employed and is shown here for Al₂O₃ NPs (**3A and B**).



Results and Discussion

For both DMPC/DiA-16 (97/3) SUVs and SLBs in water, the suspensions formed from SASUV/SASiO₂ ≥ 1/1 are stable, and nano-DSC indicates SLB formation (**Figure 4-Right**), which remained stable for at least a week (longest time measured). Although the individual particles cannot be observed, the fluorescence build-up, and the residual fluorescence after 2 hr and extensive rinsing of the microfluidic device with distilled water, indicate whether the lipids attach to the silica bottoms of the device. In the case of the DiA-16 labelled SUVs, there is extensive residual fluorescence on the silica, indicating that adsorption (and most probably vesicle fusion) has occurred (**Figure 4-Left**). While this is expected from many previous experiments of fusion of lipid vesicles^{11,12}, it demonstrates clearly why pure vesicles cannot be used for bioremediation: the vesicles will fuse prematurely to the silicate surface found in soils. By contrast, when the vesicles are already attached to silica (as in the case of the SLBs), they remain attached to the SiO₂, at

least on the time frame investigated here of 2 hrs, and the residual fluorescence is lower. Although lipid exchange (between 2 surfaces) can occur, it is a much slower process, which depends on temperature, pH and ionic strength.

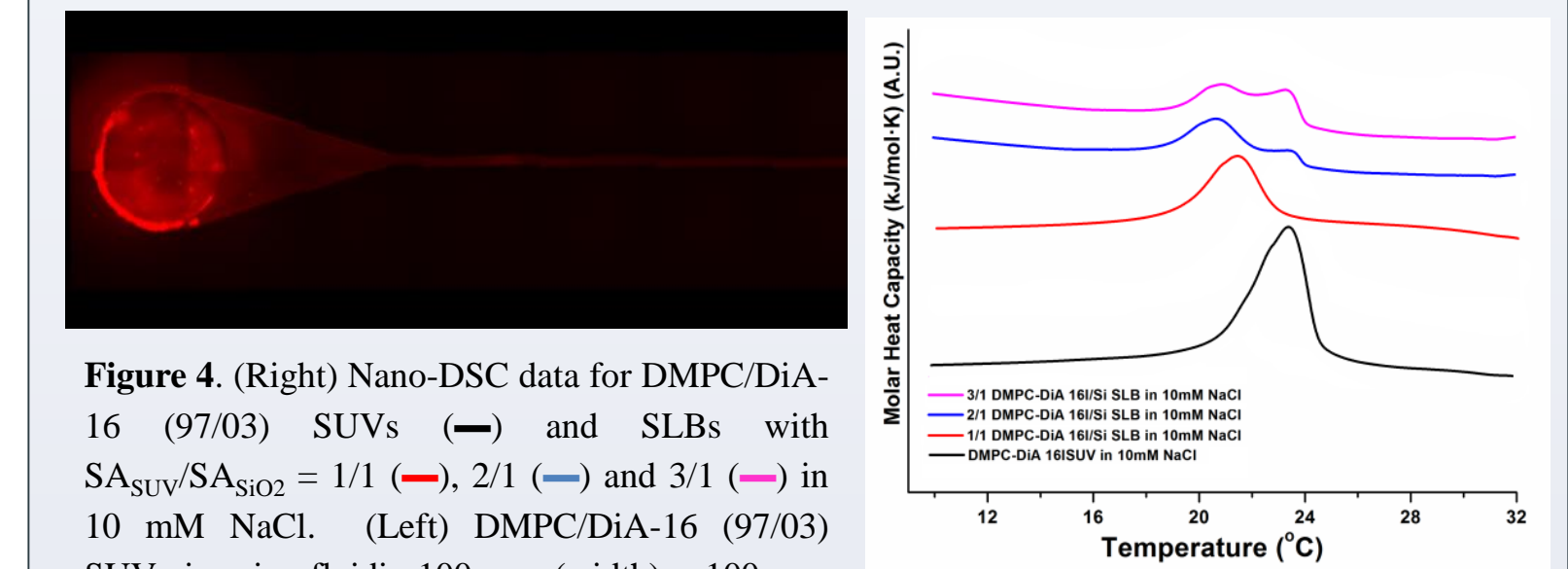


Figure 4. (Right) Nano-DSC data for DMPC/DiA-16 (97/3) SUVs (—) and SLBs with SASUV/SASiO₂ = 1/1 (—), 2/1 (—) and 3/1 (—) in 10 mM NaCl. (Left) DMPC/DiA-16 (97/3) SUVs in microfluidic 100 mm (width) x 100 mm (depth) channel near injection port.

Discussion

The adsorption of lipids on nanoparticles can endow them with anti-fouling properties and enhanced colloidal stability and thus transport, similar to that provided by pegylation. Enhanced NP mobility is also provided by stabilizing agents such as surfactants and humic acid^{30,39,71}. Polymer coatings have been shown to inhibit self-aggregation of (Ag) NPs but to enhance deposition on silica surfaces⁷², while functional groups (e.g. COOH, PEG) on polymer NP coatings had different effects on their transport through water-saturated sand columns⁷³.

Aknowlegment

Dr. Kim is thankful for financial support from Temple University as part of her start-up. The authors also thank Professor Omar Z. Fisher (Bioengineering, Temple University) for his help on the synthesis of fluorescently-labeled metal oxide nanoparticles, and Professor Mohammad F. Kiani (Mechanical Engineering, Temple University) on the imaging of the micro-model channel studies. We are also appreciative of important assistance from Samantha L. Shumlas (Department of Chemistry, Temple University) on the TEM analysis.

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