Surface Characterization of Nanoparticles for Biomedical Applications

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Statement of Purpose: Nanoparticles exhibit unique surface properties and require well-controlled surface properties to achieve optimum performance in complex biological or physiological fluids. Despite the widespread appreciation of the unique properties of high surface area nanoparticles there is a surprising lack of detailed surface characterization of these materials, especially for nanoparticles used in biomedical applications. This is in part because nanoparticles present significant challenges for surface characterization. Thus, there is a need to develop rigorous and detailed surface analysis methods for characterizing the surface of nanoparticles. Model systems with well-defined, systematic variations of surface properties are an excellent starting point for developing comprehensive surface characterization methodologies. Previously we have developed methods for quantifying the thickness and structure of carboxylic acid (COOH) terminated self-assembled monolayers (SAMs) functionalized Au nanoparticles (AuNPs) using x-ray photoelectron spectroscopy (XPS) and low energy ion scattering (LEIS). The size, shape, and size distribution of the AuNPs was determined by transmission electron microscopy (TEM). This study provides further development of these methods and applies those methods to characterizing oligo(ethylene glycol) (OEG) SAMs on AuNPs.

Methods: AuNPs with average diameters of 14 and 40 nm were synthesized using the Frens modified Turkevich method by heating a $HAuCl_4$ solution to 100˚C under nitrogen and then adding a $C_6H_5Na_3O_7$ solution. Thiol solutions were added to the AuNP solutions to produce functionalized AuNPs with the OEG SAMs. CH_3O, OH and COOH terminated OEG SAMs were prepared and those terminal groups were used to covalently attach proteins to the functionalized AuNPs. Centrifugation and resuspension, as well as dialysis procedures were used to purify the functionalized AuNPs. The purified AuNPs were deposited onto cleaned silicon wafers for analysis. XPS experiments were done using either Surface Science Instruments S-probe or Kratos AxisUltra DLD instruments. LEIS experiments were done using ION-TOF Qtac instrument. TEM experiments were done on a Phillips CM100 instrument. Control experiments were done using flat Au surfaces.

Results: TEM showed the 14nm AuNPs had a narrow size distribution (±1 nm) and were spherical. The 40nm AuNPs had a broader size distribution (±5nm) and were less spherical. Experimental conditions were identified that resulted in complete SAMs forming on the AuNPs. The XPS results for the CH_3O and OH terminated SAMs with four OEG units are shown in Figure 1. These results suggest that both OEG SAMs were more densely packed on the 40 nm diameter AuNPs compared to the 14 nm diameter AuNPs. Fourier transform infrared spectroscopy (FTIR) experiments shows the methylene backbone groups are well-ordered on all gold surfaces, but the OEG groups are more ordered on the 40nm diameter AuNPs. LEIS experiments confirmed this difference, showing the OEG SAMs on the 14nm AuNPs were several angstroms thinner than on the 40nm AuNPs. Special XPS data analysis methods were developed to further characterize SAM covered AuNPs. Simulated Electron Spectra for Surface Analysis (SESSA) was used for this purpose. Quantities such as SAM density, thickness, surface roughness, etc. were tuned in SESSA to optimize agreement between simulated and experimental XPS compositions. The SESSA results also show the OEG SAMs are several angstroms thinner on the 14nm AuNPs, with most of this difference in the OEG portion of the SAM.

Substrate	Photoelectron	OH	OMe
14 nm AuNPs	C 1s	60.3 (0.7)	60.5 (0.8)
	O 1s	15.8 (0.8)	15.5 (0.7)
	Au 4f	22.2 (0.3)	22.4 (0.4)
	S 2p	1.7 (0.2)	1.6 (0.0)
40nm AuNPs	C 1s	60.3 (1.3)	60.8 (0.8)
	O 1s	18.2 (0.8)	17.2 (0.8)
	Au 4f	20.3 (0.6)	20.6 (0.5)
	S 2p	1.2 (0.2)	1.4 (0.1)
Flat Gold	C 1s	54.0 (0.9)	54.6 (0.5)
	O 1s	14.8 (0.5)	13.6 (0.1)
	Au 4f	29.8 (0.6)	30.3 (0.3)
	S 2p	1.3 (0.1)	1.5 (0.1)

Figure 1. XPS determined elemental compositions from Au surfaces functionalized with hydroxy and methoxy terminated OEG SAMs.

Protein immobilization onto the OH terminated OEG SAMs was done using carbonyldiimidazole (CDI) chemistry. XPS detected 7 atomic % N after immobilization of Protein G onto flat Au surfaces with OH terminated OEG SAMs activated with CDI. In contrast no XPS N signal was detected without CDI activation. When the same experiments were done with AuNPs covered with OH terminated OEG SAMs similar levels of N were detected both with and without CDI activation, indicating significant non-specific adsorption and retention of Protein G on the OEG covered AuNPs.

Conclusions: XPS and LEIS methods to quantify the overlayer thickness on AuNPs have been developed. The OEG SAMs are more well ordered and thicker on the 40nm AuNPs compared to the 14nm AuNPs. Although CDI activation works well for specific immobilization of proteins onto flat OEG/Au surfaces, significant non-specific protein occurs on the OEG/AuNP surfaces.