

X-ray Photoelectron Spectroscopy as an Effective Biomolecular Binding Site Probe

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X-ray photoelectron spectroscopy (XPS) is a versatile surface characterization tool capable of probing adsorbate structure at the topmost 100 Å layers of solid surfaces [1], and can serve as a useful biomolecular binding site probe. Although analysis is performed under conditions where equilibrium is interrupted at the solid-aqueous solution interface, insight into the biomolecular interactions at the interface can still be obtained. In particular, the technique is effective for quantifying the degree of Pb(II) binding to protein structures and antioxidants, important for understanding the metal ion's interactions with protein structures [2], and developing therapeutic agents for lead poisoning [3], respectively. Also, XPS can effectively be used to measure the relative number of surface binding sites on metal oxide nanoparticles to which extracellular material can attach, important for evaluating the cytotoxicity of various, 4th period transition metal oxide nanoparticles [4].

Divalent lead (Pb) causes oxidative damage to glutathione (GSH) in biological systems, often resulting in the release of free radicals. In a study examining the relative binding of Pb(II) with antioxidants used for lead poisoning treatment, the Pb 4f core levels were deconvoluted to quantify the degree of binding to the antioxidant surface. Within two antioxidants studied, N-acetylcysteine (NAC) and N-acetylcysteine amide (NACA), that differ only in the -OH and -NH₂ functional groups between the two structures, a greater binding affinity between Pb(II) and NACA was clearly shown. XPS analysis by Chen et al. [3] indicated greater integrated peak areas of the Pb(II) complexed to NACA than to NAC, with uncomplexed Pb(II) peaks assigned at 183.4 (3.1) and 143.3 (2.8) eV for the Pb 4f_{7/2} and 4f_{5/2} photoelectrons [with full-width-at-half maxima (fwhm) in parentheses], respectively. Peak intensities not emanating from these fixed peak centers and fwhm were attributed to chemisorbed Pb(II). Comparative atomic percent chemisorbed Pb(II) on NAC and NACA were 11.2 ± 3.5% and 21.5 ± 0.9% (performed in triplicate), respectively. The XPS data complemented inductively coupled plasma mass spectrometry (ICP-MS) analysis of the supernatant, showing free NAC and NACA antioxidants in solution with increased Pb(II) concentrations added. The affinity of Pb(II) to NACA used as an antioxidant as compared to NAC nearly doubled.

Belatik et al. [2] used XPS to delineate binding of human- (HSA) and bovine serum albumin (BSA), important model proteins of the circulatory system which have numerous physiological functions, to Pb(II) under aqueous solution conditions. In the analysis of the C 1s, N 1s and O 1s core levels after Pb(II) complexation with HSA and BSA, only the XPS line shapes of the N 1s and O 1s orbitals were altered after coordination to Pb(II) while the C 1s line shapes remained unchanged. As compared to free BSA with no complexed Pb(II), the deconvoluted O 1s peak showed enhanced intensity at ca. 535 eV, indicative of the Pb(II) withdrawing electron density after complexation. A similar trend was observed for Pb(II) complexation with HSA, in which enhanced intensity was observed at ca. 534 eV for the O 1s level after complexation. Similarly, for the BSA N 1s orbital, enhanced signal was observed at 403.5 eV after Pb(II) complexation and at ca. 403 eV after Pb(II) complexation with HSA. No such trend for BSA or HSA was observed for the C 1s core level, indicating that

the Pb(II) ion interacted with only the N and O atoms within the BSA and HSA protein molecules. This finding is an important step in the understanding of the molecular mechanism of Pb toxicity.

In correlating trends in cytotoxicity with 4th period transition metal oxide nanoparticles (NPs) with physicochemical surface structure, the O 1s core levels of the NP oxides of TiO₂, Cr₂O₃, Mn₂O₃, Fe₂O₃, NiO, CuO and ZnO have been examined [4]. In the experiment, the metal oxide NP particulates were exposed to physiological aqueous solution conditions for a period of 24-hrs, which were subsequently filtered. Their surfaces were analyzed by XPS with close attention to the O 1s core levels as a way to quantitate the relative number of surface NP binding sites. These same NPs were exposed to BEAS-2B human lung cell and A549 human bronchoalveolar carcinoma-derived cell lines to measure various levels of cytotoxicity upon exposure. TiO₂, Cr₂O₃ and Fe₂O₃ had zero to minimal toxicity (close to 100% cell viability), Mn₂O₃ and NiO showed typical dose dependent toxicity (approximately 40% cell viability) and CuO and ZnO showed potent toxicity (greater than or equal to 20% cell viability). The ratio of XPS O 1s integrated peak areas were used as a means to quantify the relative number of binding sites for each type of transition metal oxide NP. The metal oxide chemical oxidation state for each oxide centered at ca. 530 eV. Higher binding energies signified chemisorbed oxygen that extracellular material, which could attach to the NP surface, typically at ca. 531 to 533 eV binding energy. In taking the relative integrated peak area ratios of the higher BE to that of the low BE of the metal oxide chemical oxidation state, these values for TiO₂, Cr₂O₃, Mn₂O₃, Fe₂O₃, NiO, CuO and ZnO were 0.56, 3.10, 0.71, 1.06, 2.33, 6.64, and 6.76 for these NPs, respectively. Relating the cytotoxicities of these materials with measured relative number of binding sites according to Spearman's rank correlation yielded a value of $\rho=0.71$. A moderately strong correlation of material NP cytotoxicity with quantifiable surface binding sites was readily revealed. The result shows that the relative number of surface binding sites on the NPs are equally as important as other factors, such as isoelectric point and atomic number of the transition metal in governing cytotoxicity.

In summary, XPS offers a promising means of examining biomolecular interactions with antioxidant, protein and nanoparticle surfaces as they interact with solution ions or extracellular material. Correlations ranging from binding of metal ions to material cytotoxic

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effects with elemental core level shift characterization arising from adsorbates adhering to molecular or compound surfaces are readily revealed using this technique.

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