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***Project title*:**
Environmental Safety and Health (ESH) Impacts of Emerging Nanoparticles and Byproducts from Semiconductor Manufacturing - Preparation and Characterization of Nanoparticles

Deliverable:
Report on the results on physicochemical and surface characteristics of Phase 2 NPs relevant to toxicity assessment

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Background:

Numerous reports published in recent years indicate a growing concern for the potential toxicity of engineered nanomaterials (Balbus et al. 2007; Nel et al. 2006; Handy & Shaw, 2007). Toxicity research is a high priority for the semiconductor industry due to the fact that some nanoparticles (*e.g.* chemo-mechanical planarization (CMP) slurry particles) are currently used in semiconductor manufacturing, and various new nano-sized materials (nanowires, carbon nanotubes, immersion lithography nanoparticles) are being considered for upcoming manufacturing processes. Predicting the potential toxicity of emerging nanoparticles (NPs) will require hypothesis-driven research that elucidates how physicochemical parameters influence toxic effects on biological systems. Of particular concern are NPs of less than 0.1 μm that would escape normal mechanisms of cellular defense (Gwinn & Vallyathan, 2006; Stern & McNeil, 2008). The intrinsic capacity of NPs to penetrate biological tissue may in itself not be the primary cause of toxicity; rather surface properties of NPs may accentuate (or minimize) toxicity. These include high specific surface area, reactive surfaces, and adsorptive surfaces for other toxic chemicals. Contaminants can also accumulate in NPs via nano-capillary condensation (Kelvin effect) in the particle pores. NPs have very high surface curvatures, engendering high surface tensions and energies that might have unique effects on living cells. Reactive radical species can have prolonged lifetimes when sorbed

onto NPs. There is a growing consensus that reactive oxygen species (ROS, composed primarily of hydroxyl radicals, hydrogen peroxide and superoxide) are a major contributing factor of NP toxicity (Gwinn & Vallyathan, 2006; Limbach et al., 2007). ROS are normally produced in and around living tissues; however, overproduction can lead to cell toxicity and loss of cell and tissue function.

The goal of this project is to characterize the potential toxicity of current and future NPs and NP-byproducts from semiconfuctor manufacturing. The information will be used to develop mechanistic hypotheses that will be applied to developing rapid toxicity assessment protocols applicable in the industrial workplace, as well as to predicting the ESH impacts of NPs based on physicochemical properties. Our hypothesis is that the size and size distribution of NPs intrinsically makes them more adsorptive to external chemicals, and these surface molecules can contribute to the observed toxic effects of NPs on cells.

Objective and key findings:

The objective of this task is to investigate the physicochemical and surface characteristics of Phase 2 NPs relevant to toxicity assessment. **Phase 2 NPs** included CMP-related primary and secondary NPs such as cerium dioxide (CeO_2), alumina (Al_2O_3), and zirconia (ZrO_2). Silica (SiO_2), was previously investigated in Phase 1 of this study. In addition to CMP-related NPs, a variety of commercially important NPs such as nano-silver (nAg), titanium oxide (TiO_2), copper(II) oxide (CuO), manganese oxide (Mn_2O_3), iron oxide (Fe_2O_3), and zero-valent iron (n-ZVI), are also being evaluated.

Experimental measurements confirmed that contaminant retention by the selected inorganic oxide NPs is compound dependent. The moisture retention affinity of the NPs decreased in the order: $CeO_2 \sim HfO_2 > SiO_2$. In agreement with literature findings, the retention of contaminants on NPs was shown to be size dependent, as indicated by the increased retention observed for HfO_2 NPs with decreasing particle size. These results indicate that small particles have a high capacity for adsorption and retention of secondary contaminants.

A multilayer transient adsorption and desorption model was developed that could effectively evaluate the maximum capacity, the saturated surface concentration, and the fractional coverage of different NPs. The model can also be utilized to estimate the adsorption and desorption activation energies for chemisorption and physisorption of different NPs.

Additional work focusing on the stability of NP dispersions in various biological media commonly utilized in toxicity testing confirmed significant NP aggregation. Polyacrylate dispersants and proteins were shown to be effective in minimizing NP aggregation in some types of biological media.

Method of Approach

The nanomaterials selected for study have been classified in two categories, namely, Phase I NPs and Phase II NPs. **Phase 1 NPs** comprise hafnium dioxide (HfO_2) NPs used in immersion photolithography, and silicon dioxide (SiO_2) NPs utilized as abrasives in CMP slurries, while **Phase 2 NPs** include other types of CMP-related primary and secondary NPs such as cerium dioxide (CeO_2), alumina (Al_2O_3), and zirconia (ZrO_2). In addition, a variety of commercially important NPs such as nano-silver (nAg), titanium oxide (TiO_2), zero-valent iron (n-ZVI), manganese oxide (Mn_2O_3), iron oxide (Fe_2O_3), and copper(I) oxide (CuO) are also being investigated. NPs from the various materials in various sizes were obtained from commercial sources. Selected physicochemical and surface characteristics of these nano-sized inorganic oxides were investigated using a variety of techniques including:

- Determination of specific area, active site density, and surface energetics for selective adsorption to assess the NP surface ability to concentrate and retain bulk contaminants.
- Transmission- and scanning electron microscopy (TEM and SEM) to characterize nanoparticle morphology and particle size in dry conditions.
- Particle size distribution in aqueous media using dynamic light scattering detection (DLS).
- Measurement of Zeta potential as an index of NP dispersion stability.

In addition, a multilayer transient adsorption and desorption model was developed to evaluate the kinetics of contaminant-nanoparticle interactions.

Technical Results and Data:

Dispersion of NPs in biological media

Dispersion of NPs in biological media utilized for toxicity testing is very challenging (Sager et al. 200; Schulze et al. 2008). Our results confirmed that the average particle size of inorganic oxide nanoparticles (Phase II NPs) increased sharply when diluted in biological medium used in a variety of toxicity assays. As an example, Figure 1 shows the average particle size of nano-sized CeO_2 in various media commonly used in toxicity testing. The average particle size of ceria at acidic pH 4.6 was ~96 nm and the zeta potential +54 mV, suggesting that the dispersion was highly stable under acidic conditions. The average particle diameter of ceria increased sharply in all biological media to values ranging from 2648–4644 nm, depending on the medium. Similar trends were observed in experiments with all nano-sized inorganic oxides tested, with the exception of silica.

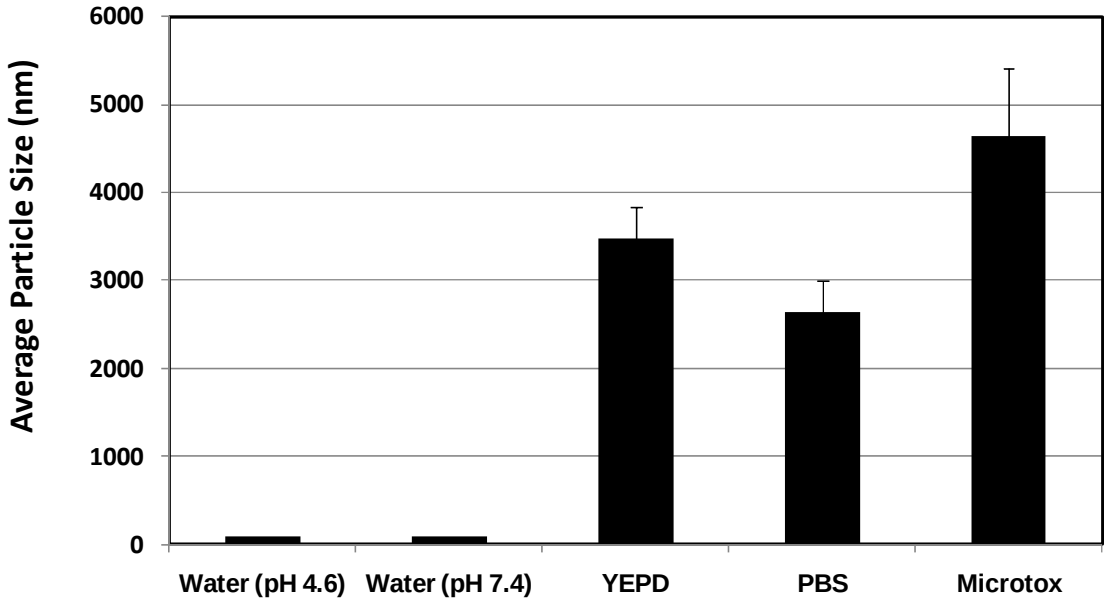

Figure 1. Average particle size distribution of CeO_2 nanoparticles (300 mg/L) in various biological media utilized in toxicity testing. YEPD (Yeast Extract Peptone Dextrose, medium used in yeast toxicity assays), PBS (Phosphate Basal Salts, biological medium used in the Dead-Live assay with human cells), Microtox osmotic medium (biological medium used in the Microtox assay, a test that relies on a fluorescent marine bacterium).

Testing the impact of material size on toxicity will require effective dispersion of the NP in the aqueous media. This project investigated various methods to functionalize NP surfaces using biocompatible ligands such poly-acrylates, thiol-terminated polyethylene glycols (PEGs), proteins, and amino acids such as lysine in order to promote the stability of NP dispersions and prevent aggregation. The results obtained indicate that polyacrylates (e.g., commercial product Dispex A40 from CIBA) are very effective in minimizing NP aggregation in biological media at concentrations which are sufficiently low to avoid biological inhibition. Selected proteins were also found to increase the stability of some NP dispersions, as will be discussed below.

Figure 2 shows the average particle size (based on light scattering intensity measurements) for different inorganic oxide NPs in water and in biological medium (yeast extract peptone dextrose or YEPD) in the presence and absence of Dispex. The results indicate a sharp increase in the average particle size of HfO_2, CeO_2 and Al_2O_3 when the materials were diluted with biological medium. Similar trends were observed for other nano- inorganic oxides (ZrO_2,TiO_2, Mn_2O_3, Fe_2O_3), and nano-ZVI (results not shown). Addition of Dispex increased the stability of the NP dispersions, resulting in a considerable decrease of the average particle size of the NPs both in water and in biological medium. Silica was the only nanomaterial which particle size was not impacted by dilution with biological medium or by Dispex supplementation.

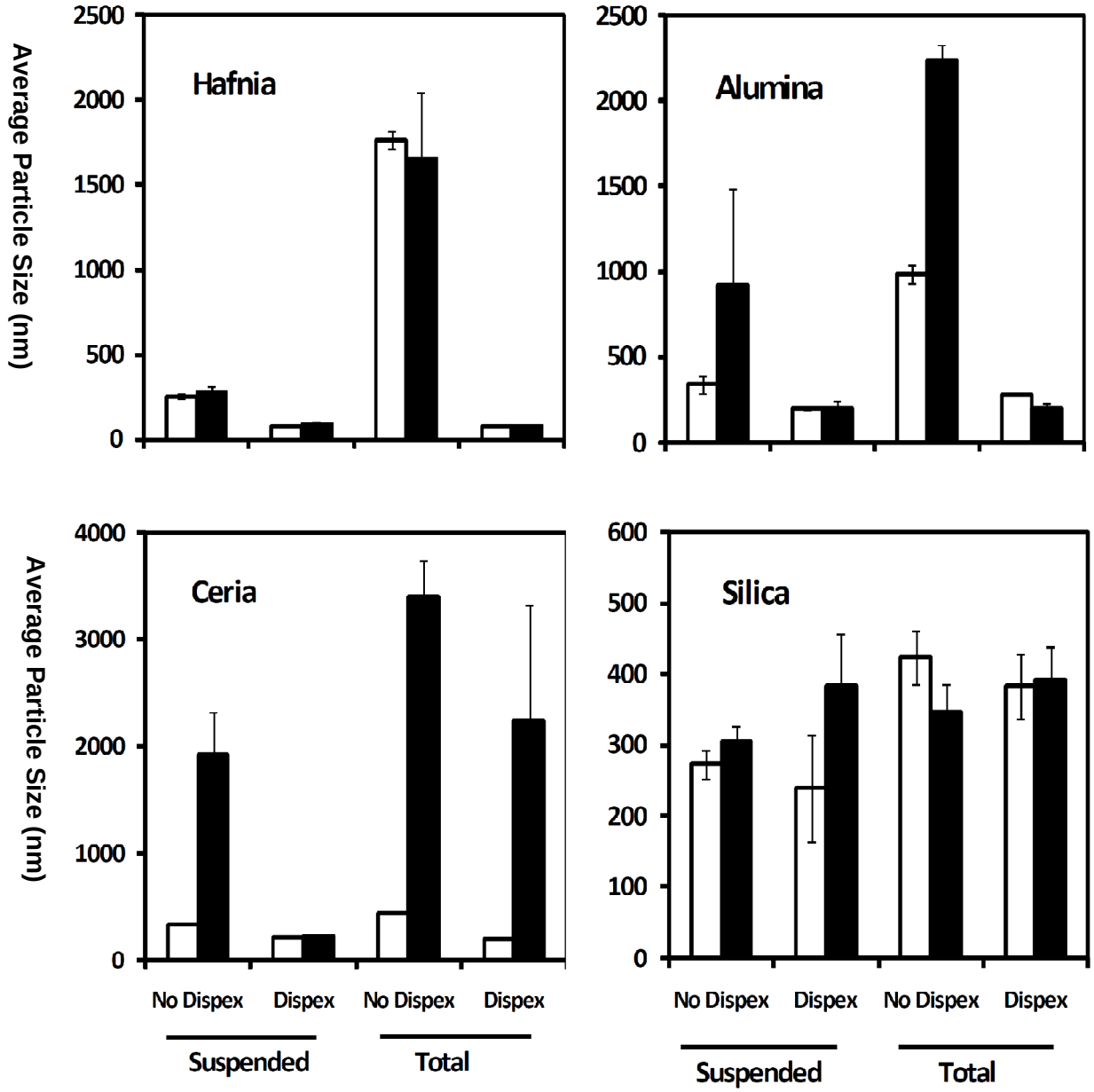

Figure 2. Average particle size of different nano-sized hafnia, alumina, ceria and silica in water (□) or biological medium (■) in the presence and absence of a dispersant (Dispex) after 10 hours of incubation. The average particle size of the total sample and the supernatant containing the suspended material is compared in the figure. The ratio NP:Dispex was 10:1 (w/w). The pH of the samples in biological medium and water was 5.5±0.2 and 4.8±0.5, respectively.

Colloids with high zeta potentials (negative or positive) are more stable than colloids with low zeta potential (or close to zero) that tend to aggregate. Zeta potential measurements (data not shown) were consistent with the particle size distribution data. In general, suspensions of HfO_2, CeO_2 and Al_2O_3 NPs supplemented with Dispex showed high zeta potential values, while NPs suspensions lacking Dispex showed values close to zero. Zeta potential values for all silica samples were high (negative), suggesting that the colloidal dispersions were stable even in the presence of biological medium.

The stabilizing effect of Dispex is also illustrated in Figure 3, which shows the average percentage of NPs determined in the supernatant (*i.e.*, suspended inorganic oxide) after 10 hours of incubation in water or biological medium in the presence and absence of Dispex. The results confirmed the presence of large fractions of the NPs in the supernatant of all samples amended with Dispex which is in agreement with particle size and zeta potential measurements.

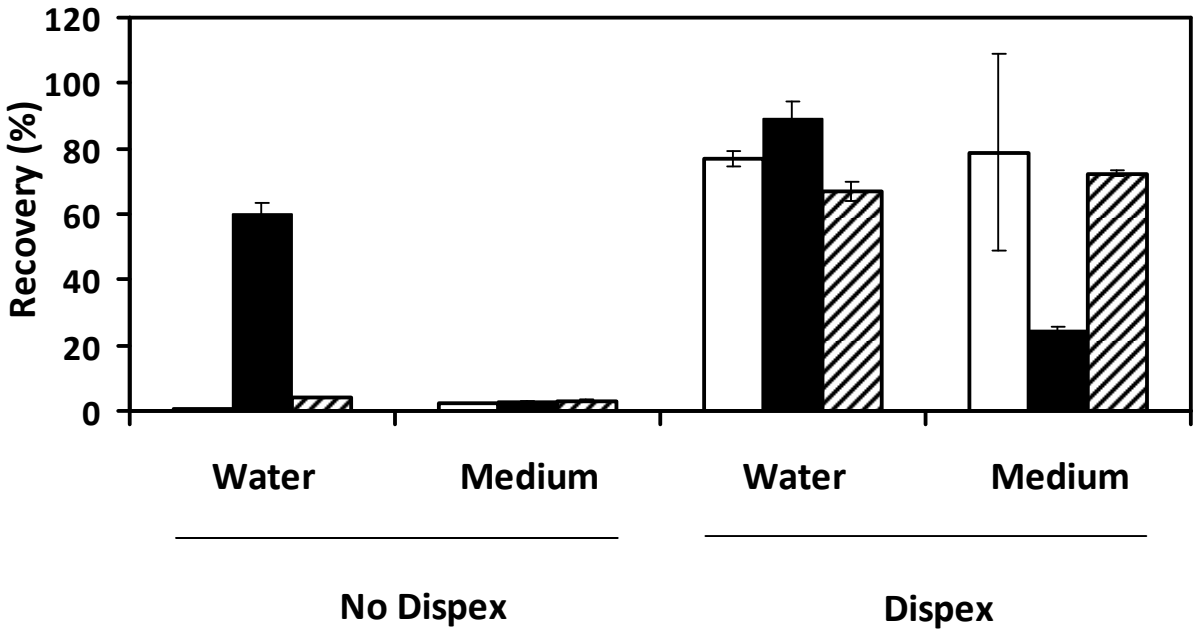

Figure 3. Impact of Dispex supplementation on the stability of inorganic oxide NPs in water and in biological medium. The figure shows the percentage of NP present in suspension (*i.e.,* not settled) after 10 hours. Alumina (□); ceria (■), and hafnia (cross hatched block).

Proteins were also very effective in increasing the stability of some nanoparticles. Figure 4 shows the average particle size and zeta potential of alumina NPs in MEM (Minimum Essential Eagle Medium) medium containing varying concentrations of fetal bovine serum (FBS). MEM is the medium utilized by our research group to evaluate NP cytotoxicity using the xCELLigence

system (Roche), a probe-free, real time cell monitoring technology instrument that shows great promise for the rapid evaluation of NP cytotoxicity. The results obtained clearly indicate that addition of proteins would be benefitial to mininize aggregation of alumina in MEM medium. Similar results were obtained with ceria NPs (results not shown). NP stabilization by some proteins has been reported previously in the literature (Ji et al. 2010). Nonetheless, some proteins have also been found to have a strong destabilizing effect on NP dispersion. In a recent study, Limbach and coworkers (2005) demonstrated that oxide NP dispersions (*i.e.*, CeO_2, TiO_2, SiO_2, ZrO_2, CuO, Fe_2O_3, and Al_2O_3) became instable in the presence of a protein-rich culture medium containing 10% fetal calf serum and dipeptides (GlutaMax). Similarly, Rezwan et al. (2005) observed a considerable decrease in the zeta potential of metal oxide colloids following adsorption of proteins (*i.e.*, bovine serum albumin and lysozyme). Electrostatic interactions protein-NP are implicated in the (de)stabilization of NP dispersions by proteins, therefore, the isolectric point of both materials will be of importance.

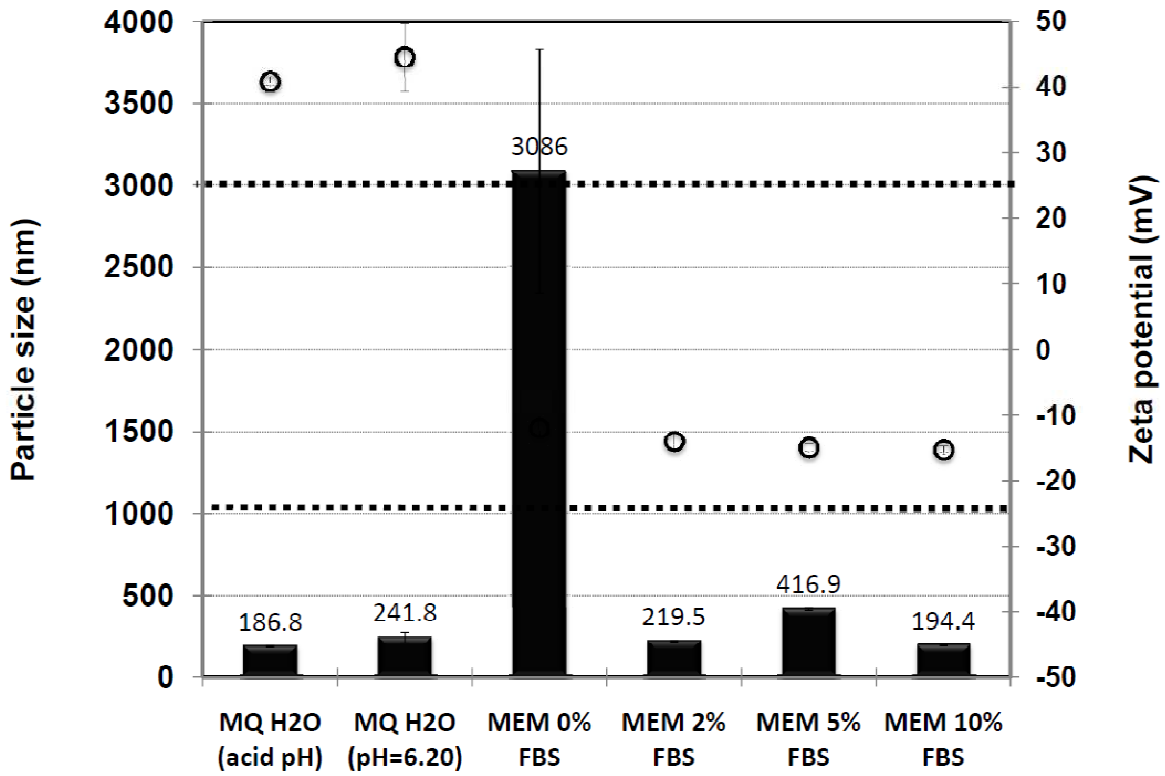

Figure 4. Impact of increasing protein (FBS= fetal bovine serum) supplementation on the stability of alumina NPs in MEM biological medium. The figure shows the average particle size (filled blocks) and zeta potential (○) determined after 10 hours. The dotted lines delineate the region (-25 to 25 mV) characterized by low NP dispersion stability.

Modeling kinetics of contaminant-nanoparticle interactions

A multilayer transient adsorption and desorption model is developed to represent the kinetics of interactions between gas phase and the solid surface of NPs. The model assumes that each available site on the surface can only adsorb one water molecule, and that all sites have identical properties. The rates of adsorption and desorption follow the elementary reaction format, in which the reaction rate is proportional to the concentration of reactants and a temperature-dependent rate coefficient. Composite activation energies of adsorption and desorption are obtained by the weighted average sum of the chemisorption and physisorption activation energies. The weight factors are the fractions of total adsorbed molecules corresponding to the two types of adsorption. *I* is the specific absorbance, which is the total absorbance peak area per unit sample area. It has a linear relationship between the specific absorbance and surface concentration.

Figure 5 shows the model simulation compared with the experimental data collected from FTIR for three oxides used in semiconductor manufacturing: SiO_2, CeO_2, and HfO_2, including HfO_2 with two different sizes.

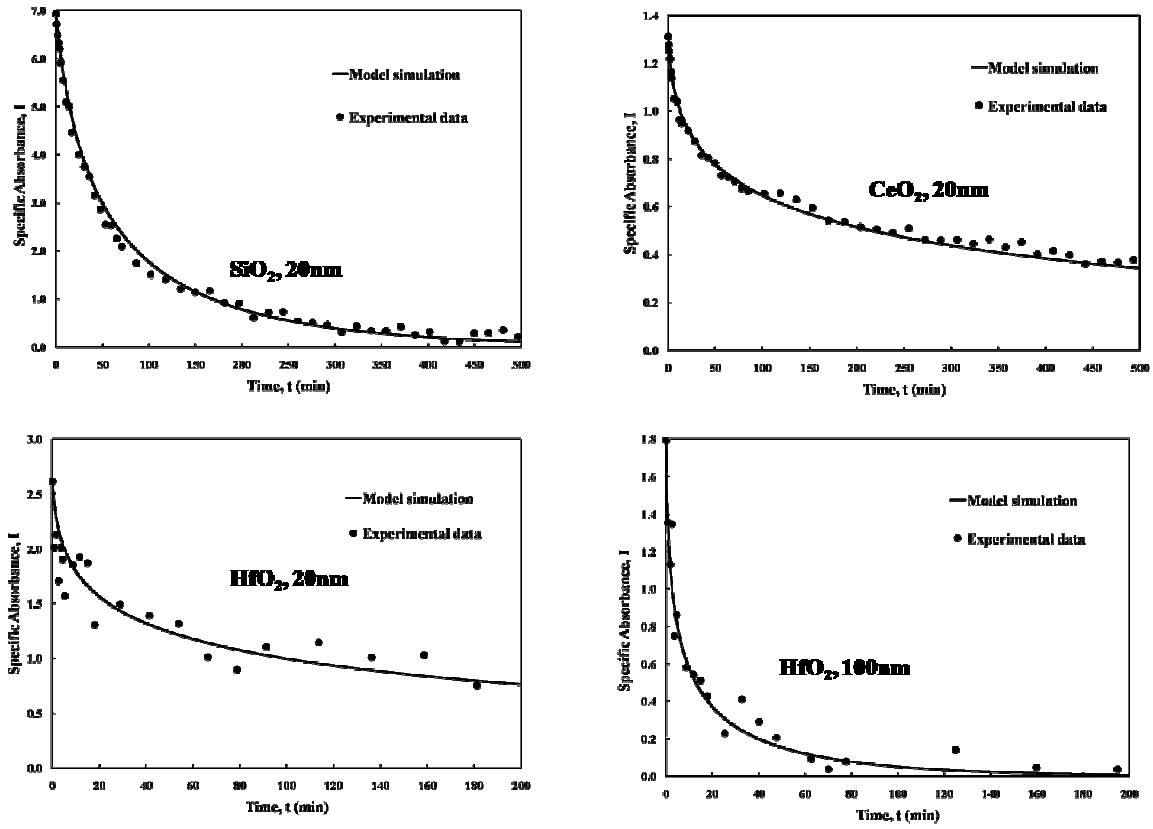

Figure 5 : Data fitting for different NPs samples

The multilayer transient adsorption and desorption model could evaluate the maximum capacity, the saturated surface concentration, and the fractional coverage of different NPs. It also can give the adsorption and desorption activation energies for chemisorption and physisorption of different NPs.

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