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## **Analysis of Binding Organic Compounds to Nanoparticles by Isothermal Titration Calorimetry (ITC)**

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### **Abstract**

Nanoparticles are a sub-classification of ultrafine particles with dimensions from one to one-hundred nanometers (nm). This study investigates replacement of an alkyl amine with an alkyl thiol on nanoparticles in a colloidal suspension by use of isothermal titration calorimetry (ITC). The effectiveness of ITC in analyzing the assembly of nanoparticles is demonstrated by determination of the enthalpy, binding constant and stoichiometry of the interaction.

### **Introduction**

Nanotechnology refers to technologies focused on development, uses and assembly of materials and devices of nanoscale dimensions ( $10^{-9}$  m) [1]. Nanomaterials and nanodevices are often conglomerates of various nanoparticles with potentially significant industrial or therapeutic applications. Nanoparticles currently under study for industrial and medical applications include buckeyballs [2], gold nanoparticles [3], iron nanoparticles [4], liposomes [5], nanocrystals [6] and carbon nanotubes [7].

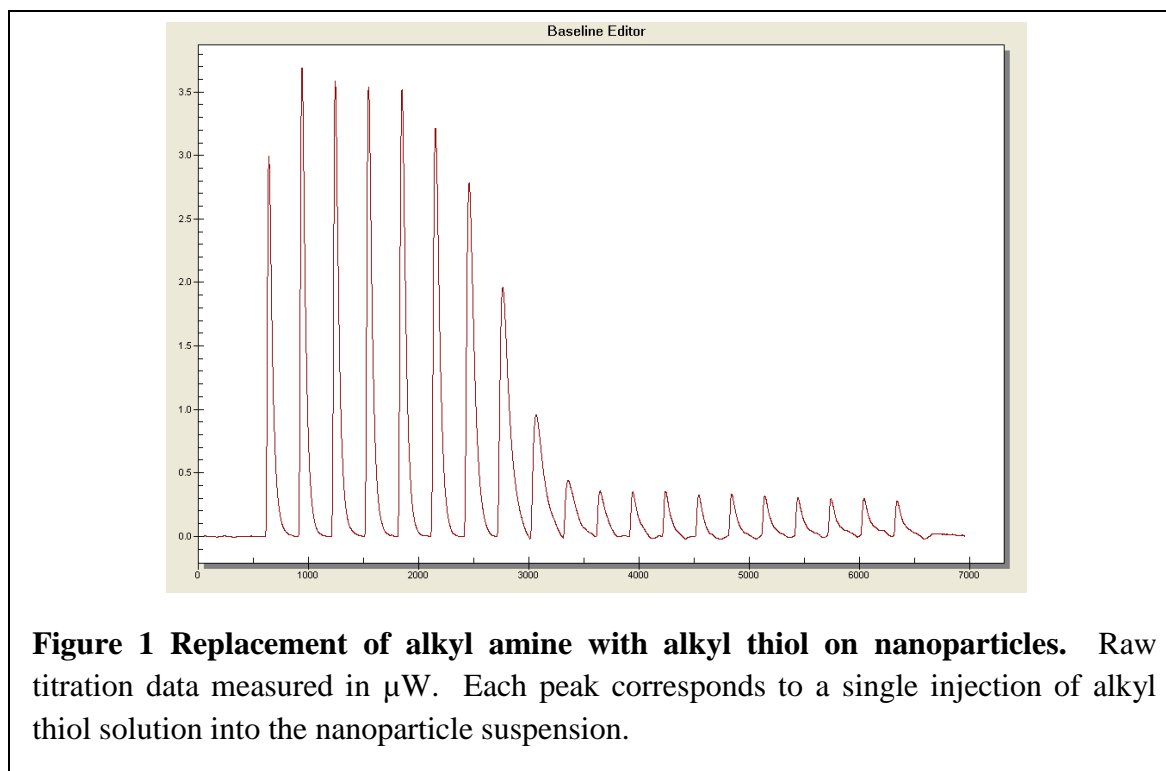
The majority of the techniques implemented to analyze nanomaterials, x-ray, neutron and light scattering, and electron microscopy, provide structural information. Calorimetry provides thermodynamic information on nanoparticle formation. To understand the thermodynamics of nanoparticle assembly, replacement of an alkyl amine with an alkyl thiol on the surface of nanoparticles in colloidal suspension was measured with a TA Instruments Nano ITC.

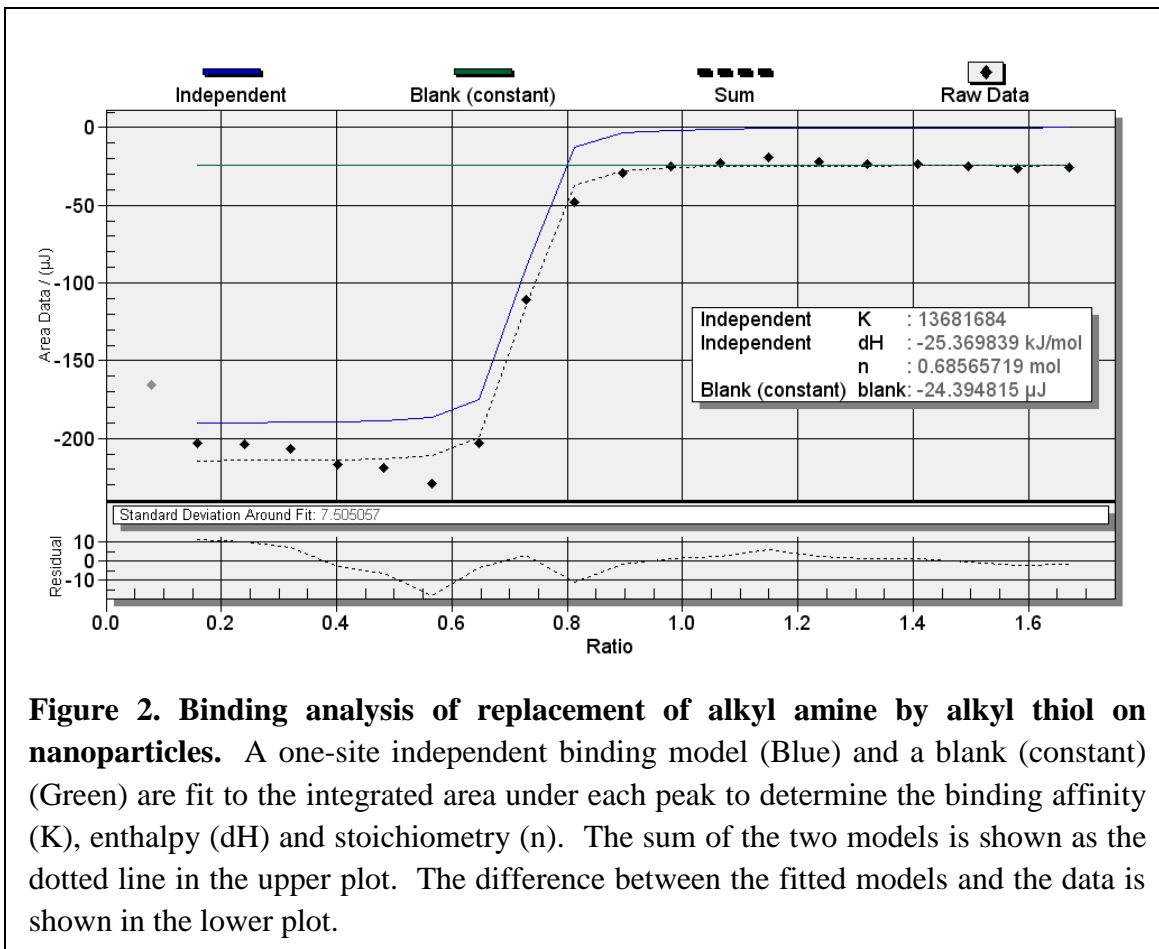
## Experimental

The nanoparticles were prepared with a monolayer of the amine conjugated to a metal-colloid core. Both the titrant (the alky thiol) and titrate (the nanoparticle suspension) were prepared in tetrahydrofuran. Titrations were performed in a Nano ITC Standard Volume with a Hastelloy reaction vessel. The reference cell was filled with water. 1.5 mM thiol solution was injected into the nanoparticle solution that contained 0.1 mM amine. Each titration consisted of twenty, 5  $\mu$ L injections at 300-second intervals with stirring speed of 350 rpm. A 300-second baseline was collected before the first injection and after the last injection. Prior to starting the titration, the calorimeter was equilibrated to a baseline with a drift of less than 100nW over a ten-minute period.

## Results and Discussion

Figure 1 shows the raw data from the titration. Upward peaks indicate an exothermic reaction. The smaller peak from the first injection is due to diffusion of titrant into the titrate during equilibration. Injection peaks 2 to 6 decrease in height from about 3.5  $\mu$ W to 3.0  $\mu$ W. Injection peaks 7 to 10 rapidly decrease, indicating binding approaching saturation. Each successive peak after the tenth is small and of similar magnitude suggesting no further binding.





The integrated area under each peak is plotted against the molar ratio of titrant to titrate in Figure 2. The first point was not included in the data analysis. Although the peaks in the raw data decrease steadily in height prior to the inflexion (Figure 1), the integrated data show the area is increasing from point 2 to point 7. This is due to broadening of the peaks, and is the result of decreased rate of alkyl amine replacement by the alkyl thiol as the reaction progresses on the nanoparticle surface. The solid lines in Figure 2 show the fit to a one-site independent binding model and blank (constant). The association constant ( $K_a$ ) of  $1.37 \cdot 10^7$  (dissociation constant  $K_d$  of 73.0 nM) corresponds to a very tight binding interaction. The enthalpy change ( $\Delta H$ ) of binding is -25.4 kJ/mol and stoichiometry of binding (n) is 0.7 mole of thiol per mole of amine. The value for the blank (constant), due to heat of dilution of the titrant, is -24.39  $\mu$ J. The standard deviation around the fit of the sum of the binding model and blank is 7.50  $\mu$ J.

## Conclusion

An analysis of the chemical details of the surface of nanoparticles is key to better understanding of the association of nanomaterials. ITC is a versatile and sensitive technique that allows for the simultaneous determination of binding affinity, enthalpy and stoichiometry of an interaction. ITC can quickly determine the thermodynamics of an interaction under native conditions, without the use of tags or labels that may otherwise interfere with binding. As an example, the displacement of an alkyl amine from a nanoparticle in suspension by an alkyl thiol was measured. The advantage of ITC over structural methods is the simplicity of the instrumentation and experiment that can be conducted without preparing crystals, complex immobilized samples, or the use of high-powered energy sources. ITC can identify the interactions between nanoparticles that are most favorable and allow for rationale design and improved assembly of nanomaterials and nanomachines.

## References

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