Characterization and Implications of Surface Hydrophobicity in Nanoparticle Fate and Transport

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Civil and Environmental Engineering in the Graduate School of Duke University

2012
ABSTRACT

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Abstract

Surface chemistry plays an essential role in determining the reactivity, bioaccessibility, bioavailability and toxicity of nanoparticles (NPs) in the environment. Processes such as aggregation, deposition and biouptake are controlled in part by the attachment efficiency, $\alpha$, between particles and the surfaces they encounter. One premise of this research is that surface hydrophobicity is a pivotal property of NP surfaces that can affect the behavior of NPs in aquatic environment and potentially decide the fate and transport of NPs. However, there are multiple challenges in the characterization of hydrophobicity for NPs. Methods developed for macroscopic surfaces or organic compounds may not be readily applied or interpreted for the case of nano-scale surfaces.

This dissertation addresses theoretical basis for applying methods to determining hydrophobicity of NPs. The use of an octanol-water partitioning method analogous to that used for organic compounds was evaluated on the basis of trends anticipated by thermodynamics, and by experimental observations. This work shows that partitioning of NPs in two phases systems, such as water and octanol, is not uniquely determined by hydrophobicity, but also influenced by surface charge and particle size. The water-oil interface rather than the bulk phases becomes the thermodynamically favored location for NP accumulation once NPs are larger than 1-10 nm and/or the surface is amphiphilic.
Nonetheless, the relative hydrophobicity of selected NPs, as characterized by adsorption of molecular probes (i.e. organic dye and naphthalene), was consistent with the macroscopic contact angle measurements and octanol-water distribution coefficients. The in-situ adsorption of these molecular probes offers the most solid grounds for measurement of hydrophobicity. Other measure of hydrophobicity or hydrophilicity such as water-affinity based methods that measure water vapor adsorption to nanomaterial powders, or immersion microcalorimetry and thermogravimetric analysis, yielded similar results to the molecular probes. However, possible physical or chemical transformations to NP surfaces during characterization by these other methods limited the use of results to infer hydrophobicity based on a rigorous thermodynamic model.

Column experiments suggested that the attachment efficiency of NPs to biofilm was generally greater for more hydrophobic NPs, though polymeric coatings might stabilize NPs against the attachment. The affinity of NPs for a variety of bacterial surfaces (i.e. different species, planktonic or biofilm, with or without extracellular polymeric substances (EPS)) of different hydrophobicities, which correlated with the quantity of proteins in EPS, was also investigated. It was found that the attachment of hydrophobic NPs increased with the hydrophobicity of bacterial surfaces, but not for hydrophilic NPs. Environmental conditions such as divalent ions and pH influenced the affinity of nanoparticle for bacterial surface by changing the bacterial surface hydrophobicity and electric double layer interaction, respectively.
Dedication

In memory of my Dad.

To TongTong, for never letting me walk alone.
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1 Introduction

Surface chemistry plays an essential role in determining the reactivity, bioaccessibility, bioavailability and toxicity of nanoparticles (NPs) in the environment. Processes such as aggregation \(^1\), deposition \(^2\), and biouptake \(^3\) are controlled in part by the attachment efficiency, \(\alpha\), between particles and the surfaces they encounter \(^1\). One premise of this research is that surface hydrophobicity is a pivotal property of nanoparticle surfaces that can affect the behavior of nanoparticles in aquatic environment and potentially decide the fate and transport of nanoparticles. Many environmental matrices that nanoparticles may encounter are hydrophobic, such as those lipid-rich sea-surface microlayer \(^4\), dense non-aqueous phase liquid (DNAPL) or light non-aqueous phase liquid (LNAPL) plume, and phospholipid membrane \(^5\). In non-hydrophobic environmental matrices such as soil, sediment \(^6\) and biofilm \(^7\), there are hydrophobic regions. The strong attractive hydrophobic interaction between hydrophobic nanoparticles and those hydrophobic matrices or regions will make them major environmental sinks for such nanoparticles. In addition, the tendency of hydrophilic nanoparticles to stay in the aqueous phase potentially enhances their mobility and probability of exposure to ecosystems and human beings. Furthermore, there is some evidence that the hydrophobicity of nanoparticles or the surface coatings may also enhance passage across lipid-rich cell membranes \(^8\).
Thorough characterization of hydrophobicity of NPs and methods for performing such characterization would therefore appear to be fundamental in the evaluation of both the potential exposure and hazards in the environment presented by nanoparticles. However, there are multiple challenges in the characterization of hydrophobicity for nanoparticles. Methods developed for macroscopic surfaces or organic compounds may not be readily applied or interpreted for the case of nano-scale surfaces. This dissertation is organized to present results that address three key questions. Can the distribution of nanoparticles in an immiscible water-oil system be used to predict hydrophobicity? Governing thermodynamic models for predicting the behavior of nanoparticles in such system are discussed and experimental evidence is presented in Chapter 4. Chapter 5 addresses the question of “What are suitable methods for characterizing surface hydrophobicity of nanoparticles?” by evaluating a spectrum of characterization techniques adapted for nanoparticles. Can the attachment of nanoparticles to bacterial surface be predicted by measure of hydrophobicity? In Chapter 6, it is demonstrated that how the hydrophobic interaction between nanoparticles and the hydrophobic region on the surface of bacteria, planktonic and attached (i.e. biofilm), determines the affinity of nanoparticles for the bacterial surface.

The following sections elaborate on the motivations for these core sections and state key hypotheses that will be tested as well as the general objectives set forth to test hypotheses.
1.1 Can the distribution of nanoparticles in an immiscible water-oil system be used to predict hydrophobicity?

1.1.1 Motivations

The partitioning between water and oil phase and the partition coefficient, $K_{ow}$ (for octanol and water), have been established as useful indicators of the hydrophobicity of non-polar organic compounds that can be used to predict concentrations in environmental compartments for the purposes of risk assessment. The fundamental assumptions in the application of this measure are: 1) solutes freely diffuse between the two liquid phases and, 2) the equilibrium distribution (i.e. the final destination) of solutes reflects the relative affinity of the solutes for each phase, suggesting a functional basis for defining the hydrophobicity of organic solutes.

However, the particulate nature of many nanomaterials poses specific challenges to interpretation of the concept of partition coefficient for characterizing hydrophobicity and predicting the physiological or environmental compartments where these materials are most likely to accumulate. Ideally, Brownian motion should be efficient in guiding nanoparticles to a preferred phase that minimizes the total free energy of the system. However, path-dependency of nanoparticle transport and the potential for nanoparticles to accumulate at the liquid-liquid interface casts some doubts on the direct extension to nanoparticles of principles previously applied for organic compounds, and, at a minimum, complicate the interpretation of such measurements. The introduction of a third phase in the form of a nanoparticle may constrain diffusion at the liquid-liquid
interface and introduces a number of additional energies that must be considered in
evaluating the thermodynamic driving force for nanoparticle transport. Particle size and
complexity of surface properties introduce additional challenges in interpreting the
meaning of $K_{ow}$ for the case of nanoparticles.

### 1.1.2 Hypotheses and objectives

With these considerations and limitations in mind, the first hypothesis to be
evaluated in this work is that surface hydrophobicity nonetheless influences the
partitioning of nanoparticles in an immiscible water-oil mixture. A related hypothesis is
that the heterogeneity of larger nanoparticles with amphiphilic surfaces favors
accumulation at the water-oil interface.

These hypotheses are examined through an analysis of the thermodynamic
principles governing nanoparticle interactions at the interface between two liquid bulk
phases and in the bulk phases. Trends anticipated from theory are examined
experimentally using a water-octanol mixture as a model water-oil system. Selected
nanoparticles of different size and surface coatings are used in partitioning experiments
allowing for a consideration of factors other than hydrophobicity such as particle size,
surface charge and partition path.
1.2 What are suitable methods for characterizing surface hydrophobicity of nanoparticles?

1.2.1 Motivations

The small size of nanoparticles excludes the possibility of conventional measurements of contact angle used to characterize macroscopic surfaces in an aqueous environment. The theoretical basis for applying methods based on the partitioning of molecular species between aqueous and hydrophobic liquids assume equilibrium conditions that are not necessarily appropriate for nano-scale matter, which may exhibit path dependency.

In addition, the design and manufacture of engineered NPs often involves the use of surface coatings for their intended applications, that are specifically design to render them more or less compatible with carrier fluids such as water or oil. The presence of surface coatings has introduced heterogeneity in the surface hydrophobicity. A heterogeneous surface that combines with the properties of the nanoparticle core complicates interpretation of interfacial phenomena; thus, suitable characterization methods for surface hydrophobicity at the nano-scale are needed.

1.2.2 Hypotheses and objectives

Considering these motivations, the second major hypothesis of this work is that the nano-scale adsorptive method, based on the adsorption of molecular probes on the surface of nanoparticles in aqueous medium, yields characterization of hydrophobicity that are consistent with either macro-scale or solute-scale methods. Moreover, a rigorous
thermodynamic interpretation of hydrophobicity can be obtained from the water-affinity based methods that characterize nanomaterial powders.

To examine the hypotheses, a variety of in-situ (i.e. nanomaterial in aqueous medium) and as-received (i.e. nanomaterial powders) characterization methods are employed to evaluate the surface hydrophobicity of selected carbon and metal-based engineered nanoparticles, and rank them by relative hydrophobicity. The experimental results from different method are compared in terms of consistency, with the intent of identifying the most appropriate characterization method. Additionally, the heterogeneity in surface hydrophobicity of nanoparticle aggregates will be studied qualitatively by selective fluorescence labeling.

1.3 Can the attachment of nanoparticles to bacterial surface be predicted by hydrophobic interaction?

1.3.1 Motivations

An important environmental interface for the fate and transport of nanoparticles is the water-solid interface. In particular, porous media, such as aquifers and sand filters in water treatment processes, consist of a great number of such interfaces. Numerous efforts have been dedicated to studying how Derjaguin-Landau-Verwey-Overbeek (DLVO) forces and non-DLVO forces such as steric interaction and hydrophobic interactions between nanoparticles and collector surface impact the mobility of nanoparticles in porous media. In this latter context, role of biofilms has been largely overlooked in extrapolating laboratory studies carried out under well-defined
physicochemical conditions to more realistic conditions where bacteria may colonize porous media surfaces. Biofilms are ubiquitous in natural and engineered environments and are commonly found in soils and at water-sediment interfaces as coatings surround particles. Surprisingly, little attention has been paid to studying the impact of biofilms on the fate and transport of ENPs in a biofilm-laden porous media. While hydrophobic interactions and steric interactions (repulsion and bridging) have been recognized as factors that affect the attachment of nanoparticles to the collector surfaces, our understanding of the factors that predict nanoparticle deposition in biofilm-coated porous media, is limited by a lack of methods to quantify hydrophobicity, in concert with a paucity of data on nanoparticle deposition to biofilms. Nanoparticles may also heteroaggregate with planktonic bacteria, and hydrophobicity is hypothesized to be an important factor in this process.

More generally, hydrophobicity may play a role determining trends in nanoparticle ecotoxicity. A systematic analysis of adsorption, distribution, metabolism and excretion (ADME) of nanoparticles by organisms is, to date, limited and hydrophobicity is likely to play an important role in these processes. The attachment of the nanoparticles onto the exterior surface of the cells or organism is the first step in the uptake of nanoparticles. In addition to be the prerequisite of nanoparticles uptake, nanoparticles attaching to the exterior surface can impair cellular functions, even for chemically inert nanoparticles not decomposing or reacting with other components in
the matrix \textsuperscript{30}. The surface acting toxins/toxicants is not a new idea to ecotoxicology either \textsuperscript{31}. Thus, studying the attachment of nanoparticles on bacterial surface is a good start in studying the bioavailability of nanoparticles.

1.3.2 Hypotheses and objectives

Based on these considerations, the third major hypothesis of this work is that hydrophobic interaction enhances the affinity of hydrophobic nanoparticles for the hydrophobic bacterial surface.

This hypothesis will be tested by evaluating the attachment efficiencies of nanoparticles of different hydrophobicities to biofilm-laden porous media through column experiments. Batch attachment experiments will be conducted to study the attachment of nanoparticles to planktonic bacterial surface with and without extracellular polymeric substances (EPS). To explore the difference in surface hydrophobicity and thus the affinity for nanoparticles, both Gram-positive and Gram-negative bacteria will be used.
2 Literature Review

2.1 Hydrophobicity and Hydrophobic interaction

2.1.1 Theoretic basis of hydrophobicity and hydrophobic interaction

The low solubility of nonpolar substances and the observed tendency of nonpolar substances to aggregate in water is known as the hydrophobic effect \(^{32-33}\). These nonpolar substances such as hydrocarbons and fluorocarbons are known as hydrophobic substances or hydrophobes, and similarly, surfaces consist of such substances are hydrophobic surfaces. This hydrophobic effect, as the name itself suggests, also describes the repulsive nature between hydrophobic substances and water \(^{34}\). Hydrophobic surfaces are not wetted by water, and a large contact angle would form upon the contact of water with such surfaces. This angle is often used to quantify the hydrophobicity, a term that denotes the physical property of hydrophobes \(^{35}\).

Hydrophobic interaction, closely related to the hydrophobic effect, describes the unusually strong attraction between hydrophobes in aqueous solution \(^{36}\). This water-mediated interaction is often stronger than that in the absence of water \(^{37}\). In contrast, hydrophilic substances prefer to be in contact with water as a result of the strong repulsion between them. Examples include strongly hydrated ions and zwitterions \(^{32}\).

The principle of the hydrophobic effect was first explicitly introduced by Irving Langmuir in 1938 \(^{38}\), followed by a landmark paper in an attempt of providing a first detailed theory of the hydrophobic effect by Frank and Evans \(^{39}\). In 1950s, Klotz \(^{40}\) and
Kauzmann were among those who first proposed that a “hydrophobic bond” was responsible for the tendency toward adhesion between hydrophobic molecules in water. Despite still being used occasionally today, hydrophobic bond was considered by some researchers in 1960s as an inappropriate description of the hydrophobic interaction due to the lack of characteristic features of chemical bonds. More and more scientists began to realize that the suspending solvent medium (i.e. water), rather than the hydrophobic substances, was a more probable origin of the hydrophobic interaction. Although many questions surrounding the origin of and thermodynamics involved in the hydrophobic effect and hydrophobic interaction remain unanswered, the development of computational methods and force measurement methods starting from 1970s has shown that the model of hydrophobic bond could not be reconciled with the physical properties of hydrophobes in water, and we are edging closer to the complete understanding of this phenomenon.

It is now widely accepted that the hydrophobic effect comes from the inability of hydrophobic molecules to form hydrogen bonds with water molecule. When hydrophobic substances are introduced into contact with water molecule, the scarcity of accommodation sites for hydrogen bonding on the surface of hydrophobes will lead to a rearrangement for the configuration of water molecules to salvage the lost hydrogen bonds. This reorientation of hydrogen-bond networks is thermodynamically
unfavorable by increasing the entropy of the system, as the existing water structure is disrupted and a more ordered structure is imposed.

However, the hydrophobic effect manifests itself differently on different length scales as illustrated in Figure 2.1. For a small hydrophobe, such as methane or argon, it excludes water molecules from a spherical volume (less than 0.5 nm in diameter) and fits into the hydrogen-bond network without sacrificing any hydrogen bonds. The change of enthalpy during the solvation is minimal, since no hydrogen bonds are broken. Thus this process is dominated entropically, and the standard entropy of solvation ($\Delta S$) is negative, as the orientational degrees of freedom of the neighboring water molecules are constrained in the presence of the hydrophobes. In addition, $\Delta S$ is proportional to the excluded volume of hydrophobes. The aggregation of two hydrophobic molecules requires fewer ordered water molecules than when they are apart, and the total free energy of the solution is lowered. Therefore, there will be a thermodynamic driving force toward aggregation for small-scale hydrophobes in water, and hydrophobic interaction at this scale is entropically driven.

On the contrary, in the case of large-scale hydrophobes, some hydrogen bonds must be broken at the interface in order to accommodate the large solute once they are inserted into water. As a result, water molecules move away from the hydrophobic surface and form an interface around it, which resembles that at a liquid-vapor interface, with one hydroxyl group of each interfacial water molecule pointing to the hydrophobic
surface. The broken hydrogen bonds at the interface result in an increased enthalpy that is proportional to the surface area of the solute. The smaller specific surface area of larger solute suggests that fewer hydrogen bonds will be missing when hydrophobes aggregate compared with when they are apart. Thus, the hydrophobic interaction at large scale is an enthalpically driven process, rather than entropically driven.

![Figure 2.1: Reorientation of hydrogen-bond network of water around hydrophobic substances of different size scale. Taken from Chandler.](image)

Although it is now generally recognized that the hydrophobic interaction is thermodynamically driven, a strong and often long-range attraction force between hydrophobic surfaces has been observed in many experimental studies. A deep understanding of the origin and nature of this attractive hydrophobic force remains elusive, despite having been the focus of a great deal of research recently. A number of force-measuring techniques have been employed to establish the hydrophobic force-distance profile, and three typical force-distance profiles are shown in Figure 2.2 for
Figure 2.2: Hydrophobic force - Separation distance profile measured by different techniques between different hydrophobic surfaces.

(a) Hydrophobic forces between stable “chemisorbed” hydrophobic surfaces measured by surface force apparatus (SFA). (b) Hydrophobic forces between “physic-sorbed” surfactant surfaces measured by SFA. (c) Hydrophobic forces between chemically silanated surfaces measured by atomic force microscopy (AFM). Taken from Meyer et al.\textsuperscript{46}

different hydrophobic surfaces with different measuring techniques. The ranges of hydrophobic attraction vary from 8-10 nm to as large as 300 nm. Numerous papers have been reported providing possible explanations for the long-range hydrophobic force such as electrostatic effects\textsuperscript{57-58}, correlated charge or dipole fluctuations\textsuperscript{48,59}, the bridging of submicroscopic bubbles\textsuperscript{60-61}, and cavitation due to the metastability of the intervening fluid\textsuperscript{62-63}. However, none of them offers plausible explanation to this attractive force over the entire range.

2.1.2 Environmental implication of hydrophobic interaction

Hydrophobic interaction lies at the heart of so many important chemical and biophysical phenomena that researchers have devoted considerable endeavor to
understanding it over the past half century. The unusual properties of hydrophobes in water arising from the hydrophobicity play a crucial role in a wide variety of chemical phenomena such as protein folding, the self-assembly of amphiphiles into micelles and biological processes such as the aggregation of amphiphilic lipids into bilayers, the gating of ion channels.

Hydrophobic interaction also plays a pivotal role in many environmental processes that determine the fate of hydrophobic contaminants in the natural and engineered aquatic environment. The transport of hydrophobic organic compounds and nanoparticles are retarded by the sediment, soil or biofilters in a wastewater treatment process, because of the hydrophobic interaction, and thus they serve as an important sink for the hydrophobic contaminants. The hydrophobic sorption of those organic compounds on dissolved organic matter, such as humic substances or nanoparticles which act as a “Trojan horse”, will greatly mobilized the hydrophobic pollutants. Bioaccumulation of hydrophobic chemicals in bacteria, algae and fungi or fish is enhanced due to the hydrophobic interaction. Furthermore, a main mechanism for the adhesion of bacteria to natural surfaces is hydrophobic interaction, and it is also responsible for the formation and maintenance of the ubiquitous biofilm.

Hydrophobic interaction, as an important type of intermolecular forces, operates between surfaces or particles in water and controls the stability of colloids.
Conventionally, there are four types of intermolecular forces that are considered to be relevant to the interaction between particles in water: (a) electrostatic double-layer (EDL) forces that are repulsive and arise when the surface is charged; (b) van der Waals (vdW) forces that are normally attractive between all molecules; (c) entropic forces such as steric interaction that can be either repulsive or attractive; and (d) structural forces that arise from the ordering of water molecules, such as repulsive hydration forces or attractive hydrophobic interaction. The first two kinds of forces were considered to be the most important and combined together in the celebrated DLVO theory as the main theoretic framework for the colloid system. However, the non-DLVO interactions, those fall in the last two types of intermolecular forces, have been investigated and shown to be equally, if not greater, important as DLVO forces in some cases. For example, the dynamic surface force apparatus is used to directly measure the force-separation distance profile between dioctadecyldimethlammonium (DODA)-coated mica surfaces, and it is shown that hydrophobic force operate at a longer range and is larger than the vdw forces (Figure 2.3). However, it is more likely that all four forces operate simultaneously in water and it is often difficult to separate the various contributions into of each force.
2.2 Quantification methods of nanoparticles

2.2.1 Quantification of fullerene-based nanoparticles

Fullerenes are one class of nanomaterials of particular interest that present challenges in measurement in an organic (oil) phase due to difficulty in differentiating between carbon sources. Quantitative methods of measuring fullerenes in environmental systems are limited at present. Methods for analysis of C₆₀ in biological and geological samples have been reported \(^{84-86}\), as well as methods for the analysis of fullerene in environmental aqueous samples \(^{87-89}\). Current methods mainly consist of two steps: extraction of fullerene from aqueous phase into toluene followed by detection. An efficient extraction method with consistently high recovery ensures the precision of the method and a good detection method secures the sensitivity and selectivity.

Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are the two methods that are widely used in the extraction step \(^{89}\). For LLE, at least one kind of salt
such as NaCl or Mg(ClO$_4$)$_2$\textsuperscript{84,90-91} was used to enhance the recovery and close to 90% of recovery was achieved. However, in these methods different types and amounts of salt yielded different recoveries, and the effect of salts in the original samples was not considered. All of these problems contributed to inconsistent recovery, thus producing variable and inaccurate measurement results. The matrix effect of common components in natural aqueous environments like humic substances has not been previously evaluated and studies that quantify the concentrations of fullerenes in actual environmental samples are scarce\textsuperscript{88}.

High-performance liquid chromatography (HPLC) has been used to isolate fullerenes from biological and aqueous samples\textsuperscript{88-89,92}. Both UV and mass spectrometry (MS) can be coupled with HPLC separation to measure C$_{60}$ fullerene\textsuperscript{88-89}, but MS could achieve a lower detection limit\textsuperscript{93}. Additionally, quality assurance and quality control issues hindered the application of these quantification methods.

The insolubility of fullerol, the hydroxylated fullerene derivatives, in toluene suggests that the same strategy for the quantification of fullerene by extraction cannot be easily extended to the analysis of fullerol. Total organic carbon concentration was measured as a surrogate for the fullerol in samples with fullerol spiked\textsuperscript{94}. Although the lack of strong absorbance peaks in the UV–visible region\textsuperscript{95} limits the application of UV/Vis spectroscopy in quantification of fullerol, the absorption peak at 400 nm was reported to correlate with the concentration of fullerol\textsuperscript{96}. Currently, analytical methods
for their quantification at sub-ppm concentrations in environmental matrices are limited, as the stability of fullerol is very low, even under mild ionization conditions. A amide-type hydrophilic interaction chromatography in combination with tandem MS (HILIC–LC–MS–MS) method was developed, and a detection limit of 0.19 pg/mL was reported. Lower detection limit was achieved by the use of ESI-MS and the detection of a specific fragment for each precursor ion.

Extraction, separation, and detection represent the general steps for the quantification of fullerene and their derivatives in aqueous samples, but the difference in polarity indicates that a combined extraction of all species is not a simple task. For the same reason, it presents challenges to chromatographic separation, as the proper mobile and stationary phase must be selected for different separation purposes.

2.2.2 Quantification of silver and gold nanoparticles

Inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma atomic/optical emission spectroscopy (ICP-AES or ICP-OES) are the most widely used techniques to quantify silver nanoparticles because of their high speed, precision and sensitivity. ICP-MS is more favored than ICP-AES/OES due to its high sensitivity and selectivity. Samples are usually pre-digested before analyzed, as the presence of particles may block sample tips and the existence of ligands or other organic matters may interfere the accuracy of the measurement. To distinguish the nanoparticulate silver and ionic silver, a pre-separation process is always needed. Cloud-
point extraction (CPE)\textsuperscript{100}, field-flow fractionation (FFF)\textsuperscript{101-102}, hydrodynamic chromatography (HDC)\textsuperscript{103}, counter-current chromatography (CCC)\textsuperscript{104}, density-gradient centrifugation\textsuperscript{105}, tangential flow ultrafiltration\textsuperscript{106}, dialysis\textsuperscript{107} have been reported to successfully separated nano-silver from Ag\textsuperscript{+}. Recent progress in the ICP-MS working in single-particle-detection mode has enabled us to detect Ag nanoparticles and Ag\textsuperscript{+} selectively without pre-separation, provided that nano-silver suspensions are sufficiently diluted\textsuperscript{108}.

In addition to these traditional techniques, some novel methods have also been developed such as those based on fluorogenic and chromogenic probes as reported by Ahn \textit{et al}\textsuperscript{109} and Tseng \textit{et al}\textsuperscript{110}, and electrochemical electrodes-based technique\textsuperscript{111}.

The quantification of gold nanoparticles can also be achieved by ICP-MS or ICP-AES/OES, but the acid pre-digestion for Au is more time-consuming due to the use of aqua regia\textsuperscript{112}. Similar separation techniques as those used for nano-Ag particles can be applied to nano-Au particles. Furthermore, diffuse optical spectroscopy\textsuperscript{113}, flow cytometer\textsuperscript{114}, and UV/Vis spectroscopy\textsuperscript{115} have been shown to be serviceable quantification techniques for gold nanoparticles. Costa \textit{et al} measured the concentration of Au nanoparticles indirectly, by relating it with their catalytic properties toward hydrogen formation\textsuperscript{116}.
2.3 Partitioning in an immiscible water-oil system

The water droplet contact angle measurement is efficient in evaluating the macroscopic hydrophobicity; while at the molecular length scale, its application is impossible. Because the hydrophobic effect is reflected in the low solubility of hydrophobes, the solubility-based partition coefficient is a potentially better indicator of microscopic hydrophobicity. Therefore, the application and interpretation of $K_{ow}$ in characterizing hydrophobicity and ultimately the bioavailability of organic compounds is of interest. The theoretic basis for interpreting the partition coefficient is predicated on the notion that when equilibrium is reached at the end of partitioning process, the total free energy of the system has achieved a minimum. To arrive at this thermodynamically equilibrium, the distribution of solute between phases will reflect where the solvation free energy is the lowest, that is the phase the solute has the most affinity for. The partition coefficient of small molecules such as organic compounds is easily interpreted as an indicator of hydrophobicity, as there is reversibility of the partitioning that correctly reflects the relative affinity between solute and water. However, when size gets larger, the partitioning behavior of solutes becomes more complicated. Firstly, as discussed previously, size of solutes plays an essential role in the configuration of hydrogen-bond network surrounding the solutes, and the solvation energy is either enthalpy-dominant or entropy-dominant depending on the size. Secondly, large solutes residing at the water-oil interface will inevitably sacrifice part of the existing water-oil
interfacial energy, which cannot be neglected when calculating the total solvation energy. The emergence of these two complications has led to some interesting research, but the validity of using partition coefficient to quantify the hydrophobicity of larger solutes is a debate that the current work seeks to inform.

### 2.3.1 Partitioning between two liquid phases

Binks \(^{117}\) provided a simple thermodynamic model, making use of interfacial tension between water, oil and solid phase and Young’s equation, to explain the partitioning of particles between two immiscible liquid phases. His work shows a relationship between partition coefficient and the contact angle as well as the water-oil interfacial tension. An extended model of Albertsson’s work in 1958 \(^{118}\), which takes the effects of gravity, particle shape and particular features into consideration, was proposed by Boucher \(^{119}\).

The total solvation free energy was obtained by evaluating the cavity formation energy and interaction energy separately using scaled particle theory (SPT), and the partition coefficients of benzene in water-heptane and water-octanol were calculated and compared to experimentally measured values \(^{120}\). Good agreement between calculated and observed values was observed. Cavity formation energy, obtained based on SPT \(^{121-122}\), offered another way to calculate total solvation energy \(^{123-124}\) without the involvement of liquid-solid interfacial energy that are not accessible in practice \(^{121}\). Lee \(^{125}\) studied the solvation free energy of methane in water, hexane, benzene, CCl\(_4\) and N\(_2\)H\(_4\).
following this approach. The evaluation of the interaction energy proves to be more complicated and leaves more questions to be answered. Wataral et al \textsuperscript{120} considered it as simple as a combination of dispersion, inductive and dipole-dipole energy. Sitkoff et al \textsuperscript{126} took electrostatic interaction into consideration; while Kalmet et al \textsuperscript{127} included the hydrogen bonding energy.

Partitioning of polystyrene latex spheres in immiscible critical liquid mixtures of 2,6-lutidine plus water was investigated by Gallagher et al \textsuperscript{128}, and a temperature-dependent partitioning behavior was observed. The size selectivity of solute partitioning across lipid bilayers was analyzed by modeling \textsuperscript{129}. Both Henry et al \textsuperscript{130} and Binks et al \textsuperscript{11} reported that the partitioning of charged particles into an oil phase was maximized near the particle’s point of zero charge. The partitioning of different engineered nanoparticles such as fullerene, fullerol and nano-silver between water and octanol was investigated by Hristovski et al, and it was observed that dissolution and aggregation of nanoparticles could change the partitioning behavior of those nanoparticles \textsuperscript{96}.

\subsection*{2.3.2 Partitioning between liquid-liquid interface and bulk phases}

By considering the change of free energy when moving particles from the liquid-liquid interface to the bulk phase, Levine et al \textsuperscript{131} pointed out the existence of an energy well that can trap particles at the interface, thus making the partitioning of particles no longer independent of distribution path. A similar energy well is also present at the water-air interface as suggested by Pieranski \textsuperscript{132}. Binks \textsuperscript{117} confirmed this energy well and
showed that, relative to the thermal energy $kT$, the magnitude of the energy well makes the attachment of particles at the interface irreversible. However, some experimental findings have suggested otherwise \textsuperscript{133}. Levine \textit{et al} argued that the negative energy well at the liquid-liquid interface must be neutralized by some positive interaction energies between particles that accumulate at the interface \textsuperscript{131}. Steric interaction was singled out as a possible candidate by de Gennes in the context of solid surfaces grafted with polymer chains \textsuperscript{134}. The repulsive interaction could also be related to electric dipole–dipole interactions \textsuperscript{135}. Electrostatic interaction is another source of repulsive interactions for charged particles at the interface \textsuperscript{136-137}. Moreover, the line tension, which is defined as the excess free energy associated to the line where three phases meet \textsuperscript{138}, could potentially play a significant role in determining the total free energy of nanoparticles at the liquid-liquid interface \textsuperscript{139}, though its effect for colloidal and larger particles may be negligible \textsuperscript{117}. In addition, compared with colloidal particles, nanoparticles are more sensitive to thermal fluctuations, due to the reduced stability at the fluid interface resulting from the size-dependent energy well \textsuperscript{140}.

There has been an increasing interest in the investigation of colloids and nanoparticles accumulating at fluid interfaces, because the self-assembly of metallic and semiconducting nanoparticles has enabled the preparation of high quality materials of specific mechanical, optical, and magnetic properties \textsuperscript{141}. The attachment of particles at fluid interfaces also has important applications in industrial processes concerned with
foams and emulsions\textsuperscript{117,142}. Meanwhile, some basic condensed matter physics problems, such as the physical behavior of two-dimensional crystals, can be studied with the help of research on the particles adsorbed at the interfaces\textsuperscript{143}. Several experimental studies of colloidal and nanoparticles at interfaces have indicated that the accumulation and stability of particles are controlled by size, surface charge, surface hydrophobicity and shape\textsuperscript{133,144-145}.

### 2.4 Characterization of surface hydrophobicity of nanoparticles

Depending on the size of the substances, characterization methods for hydrophobicity can be divided into three categories: macro-scale, solute-scale and nano-scale. Compared to their bulk counterpart, nanoparticles are often time dispersed in aqueous medium in many risk scenarios, which requires the in-situ measurement of hydrophobicity. However, there is another group of characterization methods that are based on the affinity of nanoparticles for water and applied to nanomaterial powders. A brief review of these characterization techniques is presented below.

#### 2.4.1 Macro-scale characterization method

The traditional and most widely used method for hydrophobicity characterization for macroscopic surfaces is the measurement of the contact angle. The contact angle is considered a quick, simple way of obtaining estimates of surface tension and the hydrophobicity of a solid surface. There are two kinds of contact angle developed between three phases that are often used to characterize surface...
hydrophobicity: liquid, solid and gas or two immiscible liquids and solid (Figure 2.4) 146.

For both scenarios, when $\theta < 90^\circ$, the solid substance is deemed as hydrophilic; while when $\theta > 90^\circ$, it is hydrophobic. When $\theta$ is around 90°, the solid substance can be attractive to both hydrophobic and hydrophilic surface, thus it is considered as amphiphilic.

![Diagram](image)

**Figure 2.4: Different scenarios of contact angle.**
(a) Contact angle of a sphere solid particle form at the water-oil interface; (b) Contact angle of a sphere water drop form at a flat solid surface in the air.

The most common method of measuring the contact angle is to observe a sessile drop with a telescope or microscope147, or the Wilhelmy plate method 148. These methods work effectively for macroscopic, flat solid surfaces, but are unsuccessful when applied to spherical surfaces like nanoparticles. Several methods have been proposed for the measurement of the contact angle of NPs, such as Wilhelmy film balance 149, heat flow immersion microcalorimetry 150, gel trapping coupled with scanning electron microscopy (SEM) 151, and the atomic force microscopy (AFM) 152. However, most of them are not directly measuring the contact angle, but calculating contact angle by fitting
Experimental data to the thermodynamic or optical model. Finding reproducible contact angles is often difficult in any system as there are too many parameters to control \(^{153}\); so that it is not ideal for characterizing the in-situ surface hydrophobicity of nanoparticles. In addition, other factors such as particle size, surface roughness, surface heterogeneity and shape all affect the contact angle measurement \(^{154}\), which significantly limits the use of contact angle as a measure of surface hydrophobicity of nanoparticles.

### 2.4.2 Solute-scale characterization method

The partitioning of solutes between different phases serves as a useful indicator for the relative affinity of the solutes for each phase, and the idea of using the partition coefficient between immiscible water and oil phases as a measure of hydrophobicity has been adopted for more than half century. The partition coefficient is the ratio the solutes in the two reference phases at equilibrium \(^{155}\). A fundamental assumption in interpreting partition coefficient is that the solutes freely diffuse between the two reference phases yielding an equilibrium distribution that reflects the relative affinity of the solutes for each phase \(^{10}\).

Out of the two reference phases, water is always selected as one of them, representing the hydrophilic part. Many organic solvents can be used as the other candidate as the hydrophobic part, such as octanol, hexadecane \(^{156}\), dodecane \(^{157}\), and octane \(^{158}\). Among them, octanol is the most commonly used. The octanol-water partition coefficient \((K_{ow})\) is not only considered as a serviceable indicator of hydrophobicity of
organic compounds in environmental studies, but also a useful indicator of the environmental concentration and exposure of organic compounds as applied to traditional risk assessment. It is important for risk analysis of a wide range of inorganic and organic contaminants in terrestrial systems. In the context of marine and estuarine, nanoparticles with higher K\text{ow}, such as fullerene and carbon nanotubes, will partition into the uppermost lipid moiety of the sea-surface microlayer. The K\text{ow} of nanoparticles may partially reflect the potential for diffusive uptake of certain nanoparticles, and explain the partitioning of hydrophobic nanoparticles into lipid-rich cellular parts, as partitioning between water and octanol gives a relatively good approximation of the partitioning between the cytosol and lipid-rich cell membranes in living systems. Because K\text{ow} of hydrophobic organic compounds is usually quite large, its logarithm to the base 10, logK\text{ow}, is used more than K\text{ow} itself.

The classical and most reliable method of K\text{ow} determination is the shake-flask method, in which some of the analyte under testing are dissolved in a mutually saturated mixture of octanol and water, and then the concentrations of the analyte are measured in both water and octanol. This is the most accurate method to date for measuring the K\text{ow} of a wide range of analytes. However, this method is time consuming, and if the solute is of extreme hydrophobicity, the concentration in one phase will be very small and thus difficult to measure.
The application of partitioning and the partition coefficient at the nano-scale has already been questioned. Three physic-chemical properties are responsible for governing partition behavior of solute between two liquid phases: (1) molecular weight, (2) hydrophobicity, and (3) superficial net electrochemical charge. The obvious difference in molecular weight and size between organic compounds and nanoparticles suggests that the standard method of determining $K_{ow}$ may be flawed fundamentally at the nano-scale; though Brownian motion should be efficient in guiding nanoparticles to a preferred phase that minimizes the total free energy of the system. This might change the perspective for environmental risk assessment where the partition coefficient is used as an indicator for sediment accumulation or bioaccumulation studies.

Furthermore, as described subsequently, path-dependency of nanoparticle transport and the potential for nanoparticles to accumulate at the liquid-liquid interface or aggregate may prevent proper distribution of nanoparticles in two liquid phases of the $K_{ow}$ measurement, undermining the fundamental assumption that they can diffuse freely between the phases. The complexity of surface properties introduces additional challenges in interpreting the meaning of $K_{ow}$ for the case of nanoparticles.

The use of $K_{ow}$ as an indicator for bioaccumulation may therefore not be adequate for nanoparticles. The application of $K_{ow}$ in characterizing the environmentally relevant properties of nanomaterials may vary depending on their composition, size distribution and surface treatment. To apply the same models for the risk assessment of
chemicals to nanoparticles, the question of how the use of $K_{ow}$ could be translated into the context of nanoparticles needs to be addressed.

### 2.4.3 Nano-scale characterization method

A simple yet effective method to quantify in-situ surface hydrophobicity of nanoparticles is to measure the adsorption of molecular hydrophobic probes on the surface of nanoparticles. The hydrophobic interaction is the main driving force for the adsorption of such chemical probes to hydrophobic surfaces, thus this process is also known as hydrophobic sorption. Hydrophobic sorption often occurs in the sediment or soil where hydrophobic organic contaminants selectively adsorb to the hydrophobic zone.

Hydrophobic sorption can be utilized to quantify the degree of hydrophobicity of surfaces by selecting suitable molecular probes. Historically, Rose Bengal (RB), a xanthene organic dye, has been widely used in pharmaceutical studies to determine the surface hydrophobicity of nanoparticle drug delivery vehicles. RB shows an increased adsorption to particle surface with increasing particle hydrophobicity, which makes it an ideal hydrophobic chemical probe.

Polycyclic aromatic hydrocarbons (PAHs), such as naphthalene and anthracene, represent another set of hydrophobic chemical probes. PAHs consist of aromatic rings that make them hydrophobic, and they are shown to be prone to adsorb to hydrophobic
surfaces like fullerene, carbon nanotube, and clay compared with hydrophilic surfaces.

While the adsorption of hydrophobic chemical probes on the nanoparticle surface can be used as a relatively good indicator of the surface hydrophobicity, several limitations follow. Hydrophobic interaction, electrostatic force, hydrogen bonds as well as other chemical bonding can all contribute to the adsorption. For charged surfaces like ionic dyes and most nanoparticle surfaces in aqueous solution, the role electrostatic force plays in the interaction between particle surface and adsorbate must not be neglected. Hydrogen bonds and π-π bonds are important mechanisms for the adsorption on carbon nanomaterials. Thus when studying the adsorption of hydrophobic chemical probes, the effects of electrostatic force as well as other factors need to be investigated and control experiments are necessary to exclude the influence of those interferences.

### 2.4.4 Water-affinity based characterization method

Dynamic water vapor sorption, a gravimetric technique that measures the quantity and kinetics of water adsorbed by a solid sample, has been widely used to characterize the affinity of solids for water and moreover the pore structure of porous solid samples. According to International Union of Pure and Applied Chemistry (IUPAC), there are six types of adsorption isotherms (Figure 2.5). Type I, also known as the Langmuir isotherm, is a typical adsorption isotherm for microporous adsorbents.
Types II and III are typical adsorption isotherms nonporous materials, with type III suggesting a weaker affinity for adsorbates than type II. For mesoporous materials, types IV and V occur and exhibit hysteresis loops. And type IV indicates that the attractive force between adsorbent and adsorbate is stronger than type V. When the temperature is near the melting point for the adsorbed gas, type VI usually occurs. In general, a stronger adsorbent-adsorbate interaction is shown by type I, II, IV and VI adsorption isotherms and a monolayer of adsorbates forms on the adsorbent surface. Thus, BET theory can be applied to these isotherms, and the BET constant often serves as
an indicator of the affinity for water molecule. Therefore, numerous studies have been reported to characterize the hydrophobicity of powder particles with water vapor adsorption experiment \cite{181, 183-185}.

Microcalorimetry has been employed for a long time to study the properties of solid surfaces and how they interact with liquids. The immersion calorimetry is especially a useful technique to characterize the surface chemistry of solids \cite{186}. The immersion enthalpy of immersing a solid into a liquid is considered to relate with the affinity of the solid for the liquid. An endothermic or exothermic immersion corresponds to smaller or larger affinity, respectively \cite{187}. Thus, immersion calorimetry conducted in water can be used to describe particle’s affinity for water. Recent advancements in microcalorimeters have significantly expanded the application of immersion calorimetry, and the measurement of subtle changes with reasonable accuracy in thermal energy became possible. Therefore, many studies on characterizing hydrophobicity by immersion microcalorimetry have been reported \cite{150, 187-188}.

Thermogravimetric analysis (TGA) is another gravimetric technique that can be utilized to reveal the hydrophobicity of powder particles by differentiate physically and chemically adsorbed water from the solid surface \cite{189}, as the amount of water adsorbed on the surface is considered to be related to the affinity for water. TGA measures the weight loss of solid samples as the ambient temperature increases. The weight loss up to 100°C – 150°C is commonly defined as the physically adsorbed water due to
condensation, and the weight loss from 150°C up to 400°C - 500°C under nitrogen is defined as the chemically adsorbed water such as hydroxyl groups. Though there is still no universally accepted definition of hydrophobicity based on TGA results, Anderson et al. and Giaya et al. proposed equations to calculate the hydrophobicity using the relative quantity of physically and chemically adsorbed water.

2.4.5 Heterogeneity in surface hydrophobicity

Most surfaces in industrial and technological application are heterogeneous in terms of hydrophobicity, with patchy hydrophobic and hydrophilic sites. The degree of surface heterogeneity in surface hydrophobicity at both the microscopic and macroscopic level is crucial to the industrial application of biomolecules, colloidal particles, and surfaces. The spatial distribution of hydrophobic and hydrophilic region on the surface plays a significant role in the attachment of particles to a heterogeneous surface, which is of paramount importance in biological specificity and patterned materials formation. The water surface density was found to be considerably higher at a hydrophobic area surrounded by hydrophilic sites than it is at a homogeneous hydrophobic area in the first hydration layer. The self-assembly of proteins were influenced by surface heterogeneity. The capability of bacteria to utilize hydrocarbon substrates as carbon source was shown to be affected by the heterogeneity in hydrophobicity of the cell surface. The heterogeneous hydrophobic surface of Bacillus licheniformis BAS50 altered the surface activity of lichenysin A, a lipopeptide
biosurfactant produced by this bacteria. These studies highlight the importance of heterogeneity in hydrophobicity at nanoscopic length scales.

Since hydrophobicity is usually characterized at the macroscopic scale by contact angle, a simple model has been proposed to describe the contact angle of a heterogeneous surface that is composed of “patches” of hydrophobic and hydrophobic regions (Equation 2.1).

**Equation 2.1:** \[ \cos \theta = \chi_A \cos \theta_A + \chi_B \cos \theta_B \]

where \( \chi_A \) and \( \chi_B = 1 - \chi_A \) are the fraction of hydrophobic and hydrophilic region, respectively, and \( \theta_A \) and \( \theta_B \) are the contact angles on a surface composed purely of the hydrophobic or hydrophilic phase. However, measured contact angles are often inconsistent with theoretic predictions based on this equation. Solubility-based characterization methods such as oil-water partition coefficient only provide an average estimation of hydrophobicity over the entire surface, thus suitable methods to characterize the heterogeneity in surface hydrophobicity are needed. However, only limited number of studies has been reported on this problem. Dorobantu *et al* used AFM to measure the heterogeneity in bacterial surface hydrophobicity. Contact angles were measured on surfaces consisting of alternating and parallel hydrophobic and hydrophilic strips. The heterogeneity of hydrophobicity of methylated quartz surfaces and silica polymorphs was determined by time-of-flight secondary ion mass spectrometry (ToF-SIMS) and adsorption microcalorimetry, respectively. Molecular
dynamics simulation was also employed to characterize the heterogeneity in surface hydrophobicity \cite{196,209}. Selective labeling hydrophilic region on the surface of odium zeolite particles coated by alkylsilyl groups \cite{210} and silica particles coated by different amounts of didodecyldimethylammonium bromide (DDAB) \cite{211} with fluorescein isothiocyanate (FITC) was demonstrated to be another useful technique to reveal the heterogeneity in surface hydrophobicity with fluorescence microscopy.

### 2.5 Nanoparticle and bacterial surfaces

#### 2.5.1 Bacterial surfaces

##### 2.5.1.1 Surface structure of bacteria

Bacterial surfaces affect the diffusion efficiency of materials into and out of the cell, maintenance of shape, growth and division and cell turgor resistance \cite{212}. The bacterial surface structures of Gram-positive and Gram-negative bacteria, distinguished by Gram-staining \cite{213}, represent two main types of bacterial surface structure (Figure 2.6). The cell wall composition of Gram-positive and Gram-negative bacteria is notably different as shown in Figure 2.6. Both bacteria have a peptidoglycan layer, but this peptidoglycan layer is much thinner on the surface of Gram-negative compared to Gram-positive. The cell wall of Gram-negative bacteria has an additional phospholipid bilayer membrane, with the peptidoglycan layer sandwiched between the outer and inner membrane.
The composition of the outer phospholipid membrane is very similar to that of the cytoplasmic membrane. Peptidoglycans consist of sugars and amino acids, and they form a mesh-like layer wrapping around the cytoplasmic membrane of bacteria. Lipopolysaccharide (LPS), like phospholipids, has multiple fatty acid chains connected to the backbone structure. LPS strongly binds divalent cations, and has a hydrophobic membrane-anchoring region, lipid A. Since the peptidoglycan layer and outer membrane constitute the outermost cell surface of Gram-positive and Gram-negative bacteria, respectively, a variety of surface properties, chemical heterogeneities and
structural features can be found on both bacterial surface. For example, the surface of Gram-negative bacteria is generally more hydrophobic than that of Gram-positive bacteria, resulting from the hydrophobic region lipid A in LPS; though there are also some hydrophobic regions on the peptidoglycan layer. Bruinsma et al. used a membrane filter covered by bacteria to measure the water droplet contact angle and showed that the Gram-negative Pseudomonas aeruginosa was more hydrophobic compared with Gram-positive Staphylococcus aureus.

2.5.1.2 Extracellular polymeric substances (EPS)

EPS, as a complex mixture of polymers, have been an integral part of different forms of bacteria, such as pure cultures, activated sludge, and biofilms. They have also been referred to as glycocalyx, a slime layer, capsule or a sheath. EPS are important in determining many physicochemical properties of bacterial surface such as structure, surface charge, settling and dewatering properties, and adsorption ability. A net-like structure EPS form by binding with cell protects bacteria against dewatering and toxic substances. EPS provide carbon or energy to the cells in case of nutrient shortage. They are also instrumental for the formation and maintenance of biofilm.

EPS can be divided into bound EPS and soluble EPS. Bound EPS include sheaths, capsular polymers, condensed gels, loosely bound polymers, and attached organic materials; and they are closely bound with bacteria. Those EPS that are
loosely bound with cells or even dissolved are soluble EPS, such as soluble macromolecules, colloids, and slimes. The main components that are usually found in EPS are polysaccharides and proteins. Humic substances is another major component, which makes up 20% of the total mass. In addition, some other components, like lipids, nucleic acids, uronic acids and inorganic components, have also been identified in different EPS. The composition of EPS (i.e. type of macromolecules, concentration of macromolecules) can be different depending on many factors such as culture, growth phase, and environment conditions. The composition of EPS extracted from various species can also be different. EPS of Gram-negative bacteria primarily consist of neutral or anionic polysaccharides, with uronic acids or ketal-linked pyruvates as other components. Thereby, they allow cross-linking of divalent cations such as calcium and magnesium. But EPS produced by most Gram-positive bacteria are cationic. The presence of both hydrophilic (e.g. hydroxyl, carboxyl and phenolic) and hydrophobic functional groups (e.g. aromatics, aliphatics in proteins, and hydrophobic regions in carbohydrates) in EPS molecules suggests that EPS are amphiphilic. The hydrophobic fraction of EPS was found to mainly comprise proteins; while polysaccharides were the dominant macromolecules in the hydrophilic fraction of EPS.

EPS can be separated from the bacteria cells by extraction. A typical extraction procedure includes three steps: pre-treatment, extraction and purification.
extraction step is the most important one, and can be categorized as physical methods, chemical methods and a combination of both. Physical methods include sonication, high-speed centrifugation and heating. The most commonly used chemical methods are cation exchange resin (CER), ethylenediaminetetraacetic acid (EDTA) and the HCHO/NaOH methods. The extraction efficiencies of the chemical extraction methods are generally higher than those of the physical extraction methods.

The composition of EPS can be quantified by conventional chemical colorimetric methods. The polysaccharides content can be measured by the anthrone method or the phenol–sulfuric acid method, which are found to yield similar results. The Lowry method, the Bradford method, and the total N-content method are three most popular quantification methods for proteins content of EPS. A modified Lowry method was proposed by Frolund et al. to measure the humic substances. The uronic acid content can be determined by the m-hydroxydiphenyl sulfuric acid method. As for the quantification of DNA or nucleic acids, the DAPI fluorescence method, the UV spectroscopy method or the diphenylamine method is available. More and more innovative techniques become available to be applied to the analysis and characterization of the complex composition of EPS, such as environmental scanning electron microscopy (ESEM), X-ray photoelectron spectroscopy (XPS), high-performance size exclusion chromatography (HPSEC) and time-of-flight secondary-ion mass spectrometry (TOF-SIMS).
2.5.1.3 Biofilm

A biofilm is defined as “microbial cells immobilized in a matrix of extracellular polymers acting as an independent functioning ecosystem, homeostatically regulated” 251. They are ubiquitous in natural and engineered environments and are commonly found in soil, groundwater and surface water environments as coatings on the surfaces 21. Biofilm is a protected mode of bacteria, which allows them to survive under different and sometimes extreme environmental conditions 244.

Biofilms are essential components of the food chains in terrestrial and aquatic ecosystems 252; and they play an important role in the biodegradation of organic contaminants in the aquatic environment 253. The application of biofilm can be found in the drinking-water, wastewater treatment process, and the bioremediation sites 254. The formation of a biofilm may lead to biofouling 255-256 or biocorrosion 257, which has a detrimental effect to the engineered system. They also cause pollution in food processing and drinking-water distribution systems, by serving as a source of pathogens 258-259.

A wide range of different analytical methods can be used to characterize biofilms. Fundamental microbiological parameters such as total dry weight 260, biofilm thickness 261 and total cell count 262, yet useful in generally describing the state of biofilm, are not sufficient to characterize biofilm activity at microscale. Light penetration into the biofilm matrix may be hindered by the thickness of biofilm, thus the application of optical methods is limited. Commonly used methods to characterize the biofilm
structure include confocal laser scanning microscopy (CLSM) \(^{263-264}\), scanning transmission X-ray microscopy (STXM) \(^{265}\), SEM \(^{266}\), AFM \(^{267}\), photoacoustic spectroscopy (PAS) \(^{268}\), and nuclear magnetic resonance (NMR) spectroscopy \(^{269}\).

Extraction methods aforementioned to separate EPS from bacteria can also be applied to separate biofilm. The components of biofilm, such as proteins, polysaccharides, can be analyzed using similar techniques as discussed previously.

### 2.5.2 Interactions between nanoparticles and bacterial surfaces

#### 2.5.2.1 Adherence of bacteria to solid surface

Bacterial adhesion to solid surfaces has significant implication in a number of applications, ranging from biomedical materials development to water quality control technologies. In-situ bioremediation often requires the less adhesive bacteria strains, so that the transport of bacteria can be enhanced \(^{270}\). Research on the control of membrane biofouling relies on reducing the bacteria adhesion \(^{271}\). Understanding the mechanism of and the interactions responsible for bacterial adhesion is also important, as it leads to greater insight into the attachment of solid particles on the bacterial surface.

van Loosdrecht et al investigated the adhesion of *Pseudomonas aeruginosa* to polystyrene surface and by comparing the experimental results with calculation based on DLVO theory, concluded that the adhesion occurred in the secondary minimum \(^{272}\). Redman *et al* studied the adhesion of *Escherichia coli* to quartz sediment grains and made a similar conclusion that the secondary minimum was responsible for the adhesion \(^{271}\).
Since it was observed in some cases, there were discrepancies between experiment results and DLVO theory prediction, attempts were made to reconcile this problem by including non-DLVO interactions like hydrophobic interactions\textsuperscript{273-275}. Some thermodynamic models have also been proposed to address the bacterial adhesion to solid surfaces such as the Neumann equation of state approach\textsuperscript{276}, the polar-dispersion approach\textsuperscript{277} and the electro donor-electron acceptor approach\textsuperscript{278}.

2.5.2.2 Attachment of nanoparticles to planktonic bacteria

A key aspect of understanding the environment impact of nanomaterial is to learn how nanomaterials affect microorganisms. Current knowledge about nanoparticle-bacteria interactions is still limited, though it has been shown that nanomaterial and bacteria can interact intimately through attachment of nanoparticles on the bacterial surface\textsuperscript{279}, penetration of the cell membrane\textsuperscript{280} and dissolution of nanoparticles\textsuperscript{281}. Several studies have been done to investigate the attachment of nanoparticles such as fullerene\textsuperscript{279} and cerium oxide\textsuperscript{282} on bacterial surface.

Physicochemical properties of nanoparticles such as size, shape, surface hydrophobicity and coatings are believed to affect nanoparticle-bacteria interactions\textsuperscript{283}. For example, the size of nanoparticle aggregates will change the manner in which nanoparticles physically contact cell surfaces\textsuperscript{283}. A research showed that to date, only CdSe nanoparticles < 5 nm have been reported to enter cells\textsuperscript{280}. The coatings on nanoparticle surfaces are also shown to change the surface properties of nanoparticles.
and thus their interaction with bacteria\textsuperscript{284-285}. However, the effects of those physicochemical properties on the attachment of nanoparticles to the bacterial surface have not been systematically investigated.

The properties of bacterial surface itself are also suggested to affect the nanoparticle-bacteria interaction. Different bacteria species such as gram-positive and gram-negative bacteria have different surface properties such as hydrophobicity and charge, which could affect the attachment of nanoparticles on the surface. \textit{Escherichia coli} were found to be preferentially adsorbed to hydrophilic surface solid surface\textsuperscript{286}. In addition, different studies comparing the interaction between gram-positive bacteria \textit{Bacillus subtilis} and different nanoparticles with other bacteria yield completely different results, which implicates that surface hydrophobicity of both bacteria and particles are important factors affecting the attachment of nanoparticles on the cell surface\textsuperscript{287-288}. Thus systematic studies on the effect of hydrophobicity and particles size on the attachment of nanoparticles on bacteria surface are needed.

2.5.2.3 Attachment of nanoparticles to biofilms

Currently, little attention has been paid to studying the impact biofilm makes to the fate and transport of ENPs in a biofilm-laden porous media. Tripathi et al studied the transport and retention of micrometer-sized and nanosized sulfate-functionalized or carboxyl-modified polystyrene latex particles and carboxyl-modified quantum dots in biofilm-laden porous media, by column experiments using \textit{Pseudomonas aeruginosa}
biofilm coated quartz sand\textsuperscript{22}. It was concluded that the transport of these nanoparticles was retarded by the biofilm, as well as pure EPS produced by \textit{Pseudomonas aeruginosa}. \textit{Pseudomonas aeruginosa} biofilm was also used to evaluate its effect on the mobility of nanosized laponite clay particles\textsuperscript{24} and nanosized zero-valent particles\textsuperscript{25}, and a similar enhanced retention by the biofilm was observed. The transport of fullerene C\textsubscript{60} nanoparticles was retarded by biofilms formed by \textit{Escherichia coli} (E. coli)\textsuperscript{26}. The surface potential alone, thus the electrical double layer (EDL) interaction, could not explain the altered mobility\textsuperscript{22,26}. The hydrophobic interaction\textsuperscript{26} and steric interaction (repulsion and bridging)\textsuperscript{25} were proposed as candidates that affect the attachment of nanoparticles to the collector surface. The deposition of nanoparticles on biofilm-coated surfaces was shown to correlate with the composition of EPS, especially the ratio of proteins/polysaccharides\textsuperscript{24,228}, which suggests that EPS plays a significant role in the interaction between nanoparticles and biofilm.
3 Methods

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3.1 *Preparation of nanomaterial powders and suspensions*

3.1.1 Nanomaterial powders

Fullerene (C<sub>60</sub> and C<sub>70</sub>) and fullerol (C<sub>60</sub>(OH)<sub>24</sub>) were purchased from MER Corp. (Tucson, AZ). Gold nanoparticles stabilized by citrate (Au-CIT), TiO<sub>2</sub> (rutile) and TiO<sub>2</sub>-SiO<sub>2</sub> (rutile coated with SiO<sub>2</sub>) were obtained from Skyspring Nanomaterials Inc (Houston, TX). Silver nanoparticles stabilized by citrate (Ag-CIT), coated by polyvinylpyrrolidone (Ag-PVP) and gum arabic (Ag-GA) were prepared by freeze-drying, under vacuum, nanoparticle aqueous suspensions obtained from Center for the Environmental Implications of NanoTechnology (CEINT, Durham, NC) sample store.

3.1.2 Nanoparticle suspensions

Nanoparticle suspensions were prepared in ultrapure water (prepared by Barnstead Nanopure Diamond™ system with resistivity greater than 18 MΩ cm and dissolved organic carbon concentration <3 µg/L).

Aqu-nC<sub>60</sub> suspensions were prepared by the extended stirring technique<sup>289</sup>. Here, approximately 0.5 mg/mL of fullerene powder was added to ultrapure water and the solution was stirred at 500 rpm for two weeks using a magnetic stirrer. After this, the
suspension was centrifuged for 20 min at 3000 rpm. The supernatant was withdrawn and filtered through a 0.45 μm methyl cellulose ester (MCE) filter. The filtered supernatant was monitored for mean aggregate size using dynamic light scattering (DLS) and for total carbon (TC) using a TC analyzer (SHIMADZU TOC-5050A) over one month. Both the particle size and TC were found to be stable over this period of time.

The THF-nC₆₀ suspensions were prepared by solvent exchange technique. Briefly, approximately 25 mg/mL of C₆₀ powder was added to fresh THF. Any dissolved oxygen was removed from the fullerene in THF mixture by purging with nitrogen. Then it was continuously stirred stored in the dark overnight to ensure a homogeneous mixture. The mixture was then filtered using a 0.22-μm nylon filter to remove unwanted solid material. Doubly deionized water (DDW) was then added to the THF/C₆₀ filtrate (volume ratio 1:1) at a rate of 1 L/min, while being continuously stirred. Subsequently, the mixture was placed in a rotary evaporator (Buchi Rotovap, Flavil, Switzerland), and the THF was removed by evaporation at 80 °C. The final solution was filtered through a 0.22-μm microfilter, and the concentration of the THF-nC₆₀ was approximately 14 mg/L.

Because of the high solubility of fullerol in water, the fullerol suspension was obtained by adding 80 mg/L of fullerol powder into ultrapure water and then stirring the suspension by magnetic stirrer at 500 rpm for 2 hr.

Silver and gold nanoparticle suspensions were obtained from sample store of Center for the Environmental Implications of NanoTechnology (CEINT, Durham, NC,
USA). Ag-CIT solutions were synthesized by sodium citrate reduction of silver nitrate in water at reflux. Ag-GA suspensions were manufactured by using gum Arabic to stabilize the silver nanoparticle formed from the reduction of silver nitrate. Ag-PVP solutions were prepared by silver nitrate and sodium borohydride, with PVP as stabilizer. Au-CIT suspensions were synthesized by sodium citrate reduction of hydrogen tetrachoroaurate in water at reflux.

3.2 Quantification methods

3.2.1 Method development for the quantification of C₆₀ in aqueous medium

3.2.1.1 Introduction

Fullerenes, a class of carbon-based molecules with a wide variety of geometries, sizes, and derivatives, have found their way into scientific fields such as physics, chemistry, life science, and material science, as well as commercial markets like cosmetics, energy production, semiconductors, and biomedicine, due to their unique chemical, electrical and optical properties. There are many structural variations of fullerenes used in commercial products, with the smallest member being C₂₀ and the most common being C₆₀. The increasing use of fullerene nanomaterials has lead to international calls for information regarding the potential release and exposure of these materials to humans and their impact on human and ecosystem health.

One of the main challenges in the assessment of the risk posed by fullerene nanomaterials is that analytical methods for the quantification of fullerene in natural
environmental systems are limited. This is crucial in understanding the fate and transport of fullerenes in the natural environment and in defining the potential exposure. A few methods were developed for the analysis of C_{60} in biological and geological samples \(^{84-86}\) and in environmental aqueous samples \(^{87-89}\). UV/vis spectroscopy was directly used by some researchers to determine the fullerene concentrations in aqueous suspensions \(^{297-298}\). It is neither a selective nor sensitive method due to its relatively higher detection limit and it is easily biased by interferences from organics and other constituents in environment samples \(^{89}\). As for other methods, the use of organic solvent such as toluene to extract fullerene from either biological or aqueous phase is crucial and the premise for following measurement. Thereby, to improve the efficiency of extraction step to ensure that it has consistently high recovery is important for the precision of the method.

There are two widely used extraction methods: liquid-liquid extraction (LLE) and solid-phase extraction (SPE) \(^{89}\). The addition of salts to enhance the recovery is a well-know technique for LLE, and NaCl or Mg(ClO\(_4\))\(_2\) has been used by different studies to achieve close to 90% of recovery \(^{84,90-91}\). However, some limitations follow for this salt-enhanced extraction. Firstly, the recoveries are not consistent depending on the type of salts used, for example, monovalent or divalent cation. Secondly, many environmental samples have a high concentration of salts such as the seawater. These problems have led to inconsistent recoveries and inaccurate measurement results. Furthermore, the
influence of common natural organic matters, such as humic substances, on the accuracy of these quantification methods has not been studied. And the direct application in environmental samples taken from complex matrices such as surface water and treated wastewater are still to be investigated ⁸⁸. In addition to the inconsistent recovery of the extraction step, the lack of quality assurance and quality control also proves to be limiting the application of these quantification methods.

The detection of fullerene after the completion of extraction is also essential to the overall efficiency of quantification. HPLC has been widely used in separating fullerenes from biological and aqueous samples ⁸⁸–⁹². UV/vis spectroscopy serves as an efficient technique to couple with HPLC for measuring fullerene ⁸⁹; while mass spectrometry (MS) can achieve a lower detection limit ⁹³.

The purpose of this study was to establish a method to address the problems aforementioned. Unlike previous methods, in this study, fullerenes were separated from aqueous matrices and concentrated from aqueous samples without the use of additional salts or other chemical compounds; so that they could be detected by UV detector and to achieve a low detection limit which is good enough for the fate and transport study of fullerene in environment as well as environmental toxicity. The effects of common components in natural aqueous samples such as salts and humic substances on the quantification of both C₆₀ and C₇₀ were also examined. The applicability of the established method was tested with four different natural water samples. The present
study also proposed the use of C₇₀ as a surrogate standard to solve the problem of inconsistent recovery under different environmental conditions.

3.2.1.2 Experimental approaches

Water sample matrix. Water samples investigated in this work were taken from four different aqueous environmental matrices: seawater, surface water, ground water and treated wastewater. The sources and water quality parameters of these water samples were summarized in Table 3.1. The dissolved organic carbon (DOC) was measured by using a TC analyzer (SHIMADZU TOC-5050A).

Table 3.1: Summary of sources and water quality parameters of the water samples from different aqueous environmental matrices.

<table>
<thead>
<tr>
<th>Type of Water Sample</th>
<th>Source</th>
<th>pH</th>
<th>Conductivity (mS/cm)</th>
<th>Dissolved Organic Carbon (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>Sigma-Aldrich</td>
<td>7.09</td>
<td>53.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Surface Water</td>
<td>Sandy Creek tributary running through the Duke Wetland (Durham, NC.)</td>
<td>8.07</td>
<td>0.221</td>
<td>23.6</td>
</tr>
<tr>
<td>Ground Water</td>
<td>Raleigh, NC (11628 Hickory Grove Church Road)</td>
<td>7.25</td>
<td>0.0861</td>
<td>0.8</td>
</tr>
<tr>
<td>Treated Wastewater</td>
<td>Hopewell WWTP (VA, USA)</td>
<td>7.54</td>
<td>0.874</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Liquid-liquid extraction procedure. A 2 ml aliquot of the aqu-nC₆₀ stock was placed in a 50-mL borosilicate glass vial. Nano-pure water and other chemicals (e.g. MgCl₂, humic acid) were added, as needed, to a final volume of 20 mL. 20-mL of toluene was added to the vial and the water-toluene suspension was mixed by vortex mixer for 3
minutes and then placed on an orbital shaker at 200 rpm for 2 hours. After shaking, the vial contents were allowed to settle and separate for 1 hour. A 10-mL aliquot of the toluene supernatant layer was removed and concentrated to 1mL under a gentle stream of nitrogen gas. The final toluene solution was placed in a 1.5-mL amber HPLC vial for analysis by HPLC-UV/Vis.

**Solid-phase extraction procedure.** Solid-phase extractions were performed using a SPE vacuum manifold (Phenomenex, Torrance, CA, USA) with 6-mL Strata C18-E cartridges from Phenomenex. 5 mL of toluene, 5 mL of acetonitrile and 5 mL of nano-pure water were used in sequence to precondition the SPE cartridges. 100-mL of sample was passed through the SPE cartridges at a rate of 10 mL/min. The cartridges were then rinsed with 5 mL of nano-pure water and allowed to dry completely under a stream of nitrogen gas for 1 hour. Then 5-mL of toluene was added to re-wet the cartridges and allowed to elute from the cartridges after 20 minutes. Afterwards, the cartridges were eluted with another 5-mL of toluene. Both 5mL toluene extracts were collected, combined and concentrated to a final volume of 1 mL under nitrogen gas stream and transferred to 1.5-mL HPLC vials for HPLC-UV analysis.

**HPLC-UV/vis analysis.** A Varian HPLC-UV/Vis system consisting of a Varian/Dynamax pump system coupled to Prostar 330 PDA detector (Palo Alto, CA, USA) was used for the quantification of fullerene. The chromatographic separation was performed on a 4.6 mm × 250 mm Cosmosil Buckyprep-M Packed Column (Nacalai
USA, San Diego, CA, USA) in isocratic mode at a flow rate of 1mL/min with a mobile phase of 100% of toluene. The working wavelength was determined by scanning from 200 to 800 nm. Fullerene standards for calibration were prepared by adding different amounts of fullerene into 10-mL of toluene with sonication.

3.2.1.3 Method detection limits and reproducibility

The 200-800 nm scan revealed that the best working wavelength for both C\textsubscript{60} and C\textsubscript{70} was 335 nm. The standard calibration curve obtained using this wavelength indicated that the detector response to C\textsubscript{60} and C\textsubscript{70} was linear over a concentration range of 15 µg/L - 15 mg/L. Retention times for C\textsubscript{60} and C\textsubscript{70} were approximately 7.7 - 7.8 minutes and 12.5 - 12.6 minutes (Figure 3.1), respectively. The efficiency of the method may be improved by minimizing the difference in retention of C\textsubscript{60} and C\textsubscript{70}, which is worth further investigation.

![HPLC-UV/vis chromatogram of C\textsubscript{60} and C\textsubscript{70} in toluene containing 1 mg/L of C\textsubscript{60} and 0.25 mg/L C\textsubscript{70}.](image)

Figure 3.1: HPLC-UV/vis chromatogram of C\textsubscript{60} and C\textsubscript{70} in toluene containing 1 mg/L of C\textsubscript{60} and 0.25 mg/L C\textsubscript{70}. 

52
Aqu-nC₆₀ and aqu-nC₇₀ stock solutions were added to nano-pure water to prepare a series of aqueous fullerene samples with concentrations ranging from 6 to 120 µg/L. The LLE and SPE methods were applied to these samples and fullerene extracts in toluene were analyzed by HPLC-UV/Vis, as described previously. Over the range of fullerene concentrations tested, linear calibration curves were obtained with good regression coefficient (> 0.99) (Figure 3.2) for both LLE and SPE, which indicated that the initial fullerene concentration had little effect on the recovery of fullerene during the extraction step, and thus the precision of this method.

The recovery of fullerene for C₆₀ and C₇₀ using both extraction methods (Table 3.2) also supported this conclusion, as the recovery of multiple replicates at different fullerene concentrations did not show any appreciable scatter. Without the addition of salt as destabilizer, both LLE and SPE achieved similar recoveries, which were 74.7% and 75.0% respectively for C₆₀. These recoveries were higher than in other studies, and the higher recoveries obtained in this study could be due to the relatively less negative ζ-potential of the aqueous fullerene suspension used in this study compared to that of other studies. Surface charges that were closer to zero led to greater partitioning of fullerene into nonpolar organic solvents such as toluene. A higher recovery of C₆₀ was obtained than C₇₀ using SPE, a result that requires further investigation.
Figure 3.2: Calibration curve of peak area of UV/vis absorbance versus $C_{60}$ and $C_{70}$ concentration in nanopure water. (a) by LLE method; and (b) by SPE method. Error bars represent ±1 standard deviation.

The whole-method detection limits were also shown in Table 3.2. These detection limits were comparable to those reported with UV detection after separation $^{88, 92}$, but higher than those with MS $^{84, 89}$ detection. Nonetheless, the detection limits of this
method were sensitive enough for environmental toxicity studies, as some studies suggested that approximately 500 μg/L of fullerene could result in toxic effect on fish.

Table 3.2: Summary of extraction recovery and method detection limit (MDL) by LLE and SPE method.

<table>
<thead>
<tr>
<th>ζ-potential (mV)</th>
<th>LLE</th>
<th>SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extraction Recovery b (%)</td>
<td>MDL (µg/L)</td>
</tr>
<tr>
<td>Aqu/nC₆₀</td>
<td>74.7±1.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Aqu/nC₇₀</td>
<td>73.2±1.7</td>
<td>5.7</td>
</tr>
</tbody>
</table>

a MDL is defined as the sample concentration that yields a signal-to-noise ratio of greater than 3:1.

b Extraction recovery showed in this table is mean ± 95% C.L.

3.2.1.4 Effects of salts on the extraction recovery

When this quantification method was applied to environment samples, it was important to study the matrix effect of substrates widely found in natural environments, such as salts and humic substances. A key factor affecting the accuracy and precision of this method was the consistency of the recovery of fullerene extract. Thus, the effect of these matrix interferences on the recovery was crucial to the success of this method.

A series of aqueous fullerene suspensions were mixed with different concentration (0.01 ~ 0.1 M) of MgCl₂ and extracted into toluene by both extraction methods to study the effect of MgCl₂ on the recovery (Figure 3.3). The results indicated that the addition of MgCl₂ facilitated the partitioning of both C₆₀ and C₇₀ into the organic phase and readily increased the recovery. The recovery reached a maximum after 0.01
M, which might have been due to the dramatically increased ζ-potential (C$_{60}$: -4.03 mV; C$_{70}$: -3.89 mV) as a consequence of the salt added.

Other researchers have proposed that the use of salts as destabilizers $^{84,90}$ is a necessary part of the quantification method to enhance recovery. However, considering the variability in salts type and concentration (e.g. NaCl, Mg(ClO$_4$)$_2$) reported, and the fact that these salts are common components in natural waters, it is not clear that consistent results can be obtained using this method.

![Figure 3.3: Effect of MgCl$_2$ on the recovery of C$_{60}$ and C$_{70}$ for LLE and SPE. Error bars represent ±1 standard deviation.](image)

3.2.1.5 Effects of humic substances on the extraction recovery

In most aquatic systems, between 50% and 90% of dissolved organic carbon (DOC) consists of humic substances, which could potentially interfere with analytical methods for nanomaterial measurement. The effect of humic acid (HA) and fulvic acid
(FA) on the recovery of C$_{60}$ and C$_{70}$ fullerenes was studied (Figure 3.4) over a
concentration range of 1-10 mg/L (as humic/fulvic acid).

The presence of either humic acid or fulvic acid greatly decreased the recovery of
both C$_{60}$ and C$_{70}$. Decreased recovery is likely a result of the increased aqueous affinity of
fullerene with the aid of humic substances, as there is ample evidence that the
association of C$_{60}$ aggregates with humic substances facilitates the dispersion of C$_{60}$ into
water $^{301}$ and enhances stability through reduced aggregation and deposition $^{2,94}$.

A lower charge density and a relatively large hydrophobic backbone consisting
of cross-linked aromatic networks have been attributed with a greater ability of humic
acid to disperse C$_{60}$ compared with fulvic acid $^{301}$. This conclusion is consistent with the
results from our study that show that recoveries were lower in the presence of humic
acid compared with fulvic acid (Figure 3.4). Also, recovery by SPE was more sensitive to
the interference from humic substances, with the greatest difference between SPE and
LLE occurring at TOC concentrations in range of 5 - 6mg/L. In the absence of humic or
fulvic acid, similar recoveries of C$_{60}$ were achieved by LLE and SPE but SPE produced
consistently lower recoveries of C$_{70}$ compared with LLE over the entire range of DOC
investigated. However, the fact that SPE can be run automatically and consumes less
solvent $^{88}$ than LLE is an important advantage in handling larger numbers of samples.
Also, the use of SPE could avoid the emulsion problem of LLE method $^{89}$. But the low
recoveries of C$_{70}$ obtained by SPE in even these highly idealized conditions, and the
possible interferences that may occur due to the presence of small amounts of non-aqueous phase liquid in aqueous samples, suggest that SPE may not be an option for C\textsubscript{70} measurement.

Figure 3.4: Effects of HA and FA for LLE and SPE methods. (a) C\textsubscript{60}; and (b) C\textsubscript{70}. Error bars represent ±1 standard deviation.
3.2.1.6 Extraction recoveries from environmental aqueous matrices

To better understand the applicability of this method in more complex environment matrices, four different natural waters were selected to evaluate the recovery of fullerene (Figure 3.5).

![Diagram showing recovery of C_{60} and C_{70} in different water samples.](image)

**Figure 3.5: Recoveries of C_{60} and C_{70} in different water samples.**
Error bars represent ±1 standard deviation.

Complete recovery was not achieved in any of these matrices. However seawater produced the highest recoveries (approximately 90% for both C_{60} and C_{70}) due to the high salinity (>32 g/L) that destabilizes fullerene nanoparticles. There was no statistically significant difference in recoveries between surface water, ground water, treated wastewater and ultrapure water, although the conductivity and DOC concentration of these waters differed substantially, ranging from 0.221 mS/cm and 0.8 mg/L for ground water to 53.6 mS/cm and 23.6 mg/L for the surface water used in this work. This result appears to contradict the effect of humic substances on recovery described above. It is
possible that the different composition of the DOC from the surface water such as polysaccharide materials offset the role of the humic fraction of material present, and this is supported by the finding of other researchers that dissolved organic carbon in natural waters was less effective in stabilizing fullerene in water than commercially available humic substances\footnote{302}.

3.2.1.7 Use of C\textsubscript{70} as a surrogate standard for the quantification of C\textsubscript{60}

As results in the previous sections suggested, organic matter and perhaps other components present in natural waters, may interfere with extraction procedures in the measurement of fullerenes. Also, it is possible that fullerene is lost during the quantification process due to the adsorption or deposition to glassware. For example, a previous study reported losses of more that 40\% of C\textsubscript{60} from aqueous solutions with conductivity of 500 \(\mu\)S/cm over a period of 12 hours, regardless of sample-container type\footnote{84}. Field \textit{et al}\footnote{303} proposed that the introduction of a surrogate standard, which is chemically similar to the target analyte might be introduced in samples to address these problems. The similar chemical structure of C\textsubscript{70} makes it a suitable surrogate standard for the quantification of C\textsubscript{60}. Furthermore, results in previous sections have shown that the recovery of C\textsubscript{60} and C\textsubscript{70} were almost equivalent in many conditions tested by the LLE method. However, the lower recoveries of C\textsubscript{70} by SPE compared to C\textsubscript{60} ruled out the use of SPE in this surrogate standard approach.
A calibration curve was obtained by adding the same amount of C\textsubscript{70} into a series of C\textsubscript{60}-in-toluene standards, applying HPLC-UV/Vis analysis, and then constructing a linear regression of the ratio of the peak area of C\textsubscript{60} to the peak area of C\textsubscript{70} and the ratio of the concentration of C\textsubscript{60} in toluene to that of C\textsubscript{70} (Figure 3.6).

![Calibration curve](image)

**Figure 3.6: Calibration curve of the ratio of peak area of C\textsubscript{60} over C\textsubscript{70} versus the ratio of C\textsubscript{60} concentration over C\textsubscript{70} concentration in toluene.**

Error bars represent ±1 standard deviation.

The calibration curve showed a good linear fit ($R^2 = 0.9994$). To test this method, multiple aqu-nC\textsubscript{60} samples with different concentrations of MgCl\textsubscript{2}, humic acid and fulvic acid were prepared, as well as spikes of aqu-nC\textsubscript{60} in different natural aqueous samples. A constant amount of aqu-nC\textsubscript{70} suspension was added into each sample and the LLE method was applied. After HPLC-UV/Vis analysis, the concentrations of C\textsubscript{60} calculated by the calibration curve in Figure 3.6 were compared to the known numbers (Table 3.3). The calculated concentrations of C\textsubscript{60} were found to be close to the spiked concentrations, which indicated that the use of C\textsubscript{70} as a surrogate standard was plausible. Because of the
differences in structure and physical characteristics, C70 is not as widely used as C60. Analysis of aqueous samples from environmental matrices showed that the concentration of C70 is usually lower than C60. Thus, the use of C70 as a surrogate standard for the quantification of C60 in a real sample is worth further investigation.

Table 3.3: Comparison between spiked C60 concentration and calculated C60 concentration in different aqueous samples.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample Description</th>
<th>Spiked concentration of C70 (µg/L)</th>
<th>Spiked concentration of C60 (µg/L)</th>
<th>Calculated C60 concentration (µg/L) (Mean ± 95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ultrapure Water</td>
<td>300</td>
<td>300</td>
<td>298.2±3.2</td>
</tr>
<tr>
<td>2</td>
<td>Ultrapure Water</td>
<td>600</td>
<td>300</td>
<td>295.7±2.1</td>
</tr>
<tr>
<td>3</td>
<td>Ultrapure Water</td>
<td>1200</td>
<td>300</td>
<td>301.0±2.8</td>
</tr>
<tr>
<td>4</td>
<td>[MgCl2] = 0.01 M</td>
<td>300</td>
<td>300</td>
<td>302.9±1.4</td>
</tr>
<tr>
<td>5</td>
<td>[MgCl2] = 0.1 M</td>
<td>300</td>
<td>300</td>
<td>287.2±3.5</td>
</tr>
<tr>
<td>6</td>
<td>[Humic acid] = 1 mg/L</td>
<td>300</td>
<td>300</td>
<td>296.8±1.2</td>
</tr>
<tr>
<td>7</td>
<td>[Humic acid] = 10 mg/L</td>
<td>300</td>
<td>300</td>
<td>291.3±3.3</td>
</tr>
<tr>
<td>8</td>
<td>[Fulvic acid] = 1 mg/L</td>
<td>300</td>
<td>300</td>
<td>306.7±2.7</td>
</tr>
<tr>
<td>9</td>
<td>[Fulvic acid] = 10 mg/L</td>
<td>300</td>
<td>300</td>
<td>311.5±3.9</td>
</tr>
<tr>
<td>10</td>
<td>Surface Water</td>
<td>300</td>
<td>300</td>
<td>282.4±5.1</td>
</tr>
<tr>
<td>11</td>
<td>Treated Wastewater</td>
<td>300</td>
<td>300</td>
<td>287.4±4.9</td>
</tr>
<tr>
<td>12</td>
<td>Ground Water</td>
<td>300</td>
<td>300</td>
<td>301.8±3.2</td>
</tr>
<tr>
<td>13</td>
<td>Seawater</td>
<td>300</td>
<td>300</td>
<td>288.8±4.2</td>
</tr>
</tbody>
</table>

*Calculated concentration of C60 is based on the calibration curve in Figure 3.6.

3.2.1.8 Conclusions

In summary, a quantification method with higher precision and environment-relevant sensitivity for aqueous C60 samples has been developed by using liquid–liquid extraction and solid-phase extraction coupled with HPLC–UV/vis analysis, with detection limits as low as 4.2 µg/L for C60. The presence of salts and natural organic
matters in environmental samples is likely to interfere with the extraction of carbon-based nanomaterials such as fullerenes. The addition of MgCl₂ in both LLE and SPE methods increased the extraction recovery of C₆₀ from 75% to 90% while humic acids and fulvic acids decreased recovery to less than 40%. Humic acid was more effective at lowering C₆₀ recovery than fulvic acid. The recoveries of fullerenes from surface water, treated wastewater and groundwater matrices were statistically equivalent but the recovery of fullerene from seawater was >90%. Since the recovery of C₇₀ was similar with C₆₀ and C₇₀ was not as widely used as C₇₀, LLE coupled with the use of the reference surrogate C₇₀ appears to enable excellent quantitative measurements of C₆₀ concentrations in natural waters with a wide range of concentrations of salts and dissolved organic matters.

### 3.2.2 Quantification of other nanoparticle suspensions

Fullerol concentration was measured by UV/vis spectroscopy as described elsewhere. The concentration of Ag and Au nanoparticles in water phase was measured by ICP-MS with acid pre-digestion.

### 3.3 Characterization of nanoparticles

Particle size was characterized by TEM (FEI Tecnai G² Twin, SMIF facility at Duke University, Durham, NC). Hydrodynamic size was measured using dynamic light scattering with ALV CGS-3 system (ALV-GMBH, Langen, Germany). Electrophoretic mobility (EPM) measurements were performed using a Zeta Sizer Nano ZS (Malvern,
Bedford, MA), and zeta potential was calculated from the measured EPM. The specific surface area of nanomaterial powders was quantified by BET surface area analyzer with nitrogen gas.

3.4 Experimental approaches for the partitioning of nanoparticles in water-octanol system

The shake-flask method was used to measure the distribution of nanoparticles in a water-octanol mixture. Water and octanol were mixed and equilibrated for 1 day before experiments. 20 ml of octanol and 20 ml of nanoparticle suspension were mixed and shaken in separatory funnels at 50 rpm on an orbital shaker for 1 day at room temperature. The mixture was then allowed to stand for 3 h before aliquots were collected from both water and octanol phase. The concentration of nanoparticles in each phase was measured and used to calculate the distribution coefficient between octanol and water, \( D_{ow} \) (Equation 3.1). Nanoparticles at the interface were quantified as Equation 3.2. Control samples with nanoparticles added to single phase were also prepared to account for the adsorption of nanoparticles onto glassware.

\[
\text{Equation 3.1: } D_{ow} = \frac{\text{Mass of nanoparticles in octanol}}{\text{Mass of nanoparticles in water}}
\]

\[
\text{Equation 3.2: } M_{\text{Interface}} = M_{\text{Total}} - M_{\text{Octanol}} - M_{\text{Water}}
\]

The \( C_{60} \) fullerene and fullerol in octanol sample was evaporated and residual fullerene were dissolved in toluene and water, and quantified by HPLC-UV/vis spectroscopy, respectively. The nano-Ag and nano-Au particles in octanol sample
were evaporated, re-dispersed in nano-pure water, acid digested and quantified by ICP-MS\textsuperscript{112, 305}.

We carried out the experiments to evaluate the effect of pH on the distribution of nanoparticle in water-octanol system experiments by titrating the nanoparticle suspensions in the pH range of 1.5 – 11.5 with trace-metal grade HNO\textsubscript{3} and NaOH. During the experiments, the hydrodynamic size and surface charge (i.e. electrophoretic mobility) were monitored after desired pH was reached and after equilibrium of distribution was established. For the effect of ionic strength (IS) experiments, NaNO\textsubscript{3} was added in different concentration (10 – 100 mM) to change IS at pH = 7.

Successive filtration was conducted to fractionate aqu-nC\textsubscript{60} suspension into groups of different size\textsuperscript{306}. Membranes with nominal pore sizes of 800 nm (Nylaflo, Pall Life Sciences, Port Washington, NY), 450 nm (Nylaflo, Pall Life Sciences, Port Washington, NY), and 50 nm (Isopore, Millipore, Billerica, MA) were used, after rinsed with 200 mL of nano-pure water, to separate aqu-nC\textsubscript{60} stock solution into three groups: filtrate by 800 nm membrane (aqu-nC\textsubscript{60},<800), filtrate by 450 nm membrane (aqu-nC\textsubscript{60},<450), and filtrate by 50 nm membrane (aqu-nC\textsubscript{60},<50).

An enhanced dark field transmission optical microscope (Olympus BX41) was employed to visualize the distribution of gold nanoparticles near water-octanol interface. A drop of Au-CIT suspension and octanol were deposited next to each other on a glass slide, covered and then observed by the dark field microscope.
3.5 *Characterization methods for surface hydrophobicity of nanoparticles*

3.5.1 Contact angle measurement

A thin film of NPs was prepared by filtering the NP suspensions through 0.025μm Pore Size Millipore MF-Millipore Mixed Cellulose Ester membrane (Millipore, Billerica, MA). The membrane with its associated thin film of NPs was freeze dried overnight and then placed on the bottom of a glass petri dish. The petri dish was filled with octanol, and a drop of water was added to the octanol phase. The water drop settled on the NPs film, and the contact angle formed between water, octanol and the film (Figure 3.7) was measured by KRUSK Easydrop FM 40 (KRÜSS GmbH, Hamburg, Germany). All measurements were done in triplicate and angles were measured immediately after contact of the water drops with the NPs film.

*Figure 3.7: Contact angle measurement on a thin film of nanoparticles.*
3.5.2 \(K_{ow}\) measurement

The shake-flask method was used to measure \(K_{ow}\) analogous to \(K_{ow}\) measurements performed for organic compounds. The notion of \(K_{ow}\) was adopted to underscore the fact that the thermodynamic conditions for equilibrium distribution of the nanomaterial are not likely to be met due to the particulate nature of nanomaterials that imply path-dependency resulting from processes such as mass transport, aggregation and accumulation at phase interfaces. Octanol and water were premixed and equilibrated for 24 hr before use. 20 ml of NPs suspension and 20 ml of octanol were added to separatory funnels and shaken at 50 rpm on an orbital shaker for 24 hr at room temperature. The mixture was allowed to stand for 3 hr, followed by the collection of an aliquot of the aqueous phase. The concentrations of nanoparticles in the water and octanol phase were measured as discussed previously, and used to calculate \(K_{ow}\).

3.5.3 Organic dye adsorption experiment

The adsorption experiments of the hydrophobic dye Rose Bengal (RB) were performed as described previously \(^{172}\), because RB, a xanthene dye, shows an increased adsorption to particle surface with increasing hydrophobicity. This method has been used in pharmaceutical studies to determine the surface hydrophobicity of nanoparticle drug delivery vehicles \(^{169-171}\). Briefly, 20 mg/L of RB was added to each NPs suspension with a range of increasing concentration. Controls were prepared by adding RB to ultrapure water to account for the adsorption of RB to vials and centrifuge tubes. All the
samples were repeated in triplicate. After mixing and incubation in 0.1 M phosphate buffer (pH=7.4) for 3 hr, RB partitioned between NP surface and water. NPs were then separated from the supernatant by ultra-centrifugation at 185000g. The concentration of free RB in the supernatant was determined by UV/Vis spectroscopy at $\lambda=542.7$ nm. The partitioning quotient (PQ) was then calculated as the following equation (Equation 3.3).

Equation 3.3: $PQ = \frac{Mass \ of \ RB \ adsorbed \ on \ particle \ surface}{Mass \ of \ RB \ in \ water}$

Plotting of PQ against total SA of NPs resulted in straight lines. The slopes of these lines were calculated by linear regression analysis, and are considered as an indicator of the surface hydrophobicity of NPs.

3.5.4 Naphthalene adsorption experiment

For the naphthalene adsorption experiments, 10 mL of each NPs suspension in 0.1 M phosphate buffer (pH=7.4) was added to a 40 mL sample vial and capped with Mininert™ caps (Sigma-Aldrich, St. Louis, MO). For each sample, a control vial was set up following the same procedure, while using 10 mL ultrapure water instead of the NP suspension, to account for the possible adsorption of naphthalene to the glass and caps. The headspace in the vials was approximately 30 mL. Naphthalene-in-acetone stock solutions were then injected with a microsyringe so that the initial naphthalene concentration in aqueous phase was within the range of 0.05 to 1.0 mg/L. The volume fraction of acetone in aqueous phase in each vial was kept less than 0.002 to avoid possible cosolvent effects. The vials were rotated end-over-end at 20 rpm in the dark
at room temperature. After 3 days, the concentration of naphthalene in the headspace was analyzed by gas chromatography (GC) using the Shimadzu GC-2010 (Kyoto, Japan) coupled with flame ionization detector (FID). The concentrations of naphthalene in aqueous phase were then calculated based on Henry’s Law (with Henry’s Law constant for naphthalene at 25°C = 0.0197 308) and the concentrations of naphthalene in gaseous phase. All the samples were repeated in triplicate.

3.5.5 Water vapor adsorption experiment

The dynamic water vapor adsorption experiment was conducted by Q5000 SA (TA Instruments, New Castle, DE). Briefly, the powder sample and a blank were outgassed at 80°C for 5 hr and then equilibrated at room temperature for 2 hr. The relative humidity was increased from 0% to 20% with a step size of 2% and an equilibrating time of 2 hr. After the relative humidity rose to 20%, the step size was changed to 10% until the saturated water vapor pressure was reached. The weight of the sample with adsorbed water vapor was recorded by ultra sensitive thermobalance. A water vapor adsorption isotherm was then obtained from the varied weight over different relative humidity.

3.5.6 Immersion microcalorimetry experiment

The experimental set-up for immersion microcalorimetry is shown in Figure 3.8, and a conventional Tian-Calvet differential microcalorimeter was used to measure the enthalpy released/adsorbed accompanying the immersion of nanoparticle powders in
the solvent. The sample was placed in a glass ampule, outgassed overnight and then sealed. The ampule was fixed to the bottom of a glass rod and immersed into wetting liquid held by a wetting chamber made of steel. The ampule and the wetting chamber were then introduced into the calorimetric cell and wait until the thermal equilibrium was reached. Then the ampule was broke by pushing down the glass rod, immersion liquid flew into the ampule and mixed with the sample. The released thermal energy of the whole system was recorded by thermopile. The measured enthalpy change included a small energy for breaking the brittle end of ampule and the enthalpy of vaporization of the saturated vapor that has to fill the volume liberated by the lowering of the liquid level in the wetting chamber. Thus, the immersion experiments were conducted for a series of vacuumeed empty ampules with different volume to determine these energies, which were subtracted from the immersion enthalpies of nanoparticle powders.

Figure 3.8: Experimental set-up for immersion microcalorimetry.
Taken from Partyka et al. 187.
3.5.7 Thermogravimetric analysis

The TGA analysis was conducted using Q500 (TA Instruments, New Castle, DE). The nanoparticle powders were weighed before loaded to the instrument. They were heated in nitrogen by a heat ramp of 10°C/min from room temperature to 120°C and held at 120°C for 10 min. Then a heat ramp of 20°C/min was used to increase the temperature to 500°C and held at 500°C for 10 min with samples still in nitrogen. The nitrogen was switch to air subsequently, and the samples were heated to 800°C and cooled down gradually. The weight of sample was monitored by an ultra-sensitive thermobalance.

3.5.8 Selective fluorescence labeling

Modifying nanoparticles with aminopropyltriethoxysilane (APTS) labeled by fluorescein isothiocyanate (FITC) was required for characterization by fluorescence microscopy (Figure 3.9). First of all, to prepare FITC-APTS conjugate, 69 mg APTS and 5.25 mg FITC were combined together in 1 mL of absolute ethanol under a dry nitrogen atmosphere and stirred magnetically for 12 hr. FITC covalently attached to the APTS silane compound by a stable thiourea linkage. Then a liquid mixture was prepared by adding 1 mL of NH₄OH to 30 mL of absolute ethanol, and stirred for 5 min. Subsequently, 1.4 mL of NH₄OH and 3 mg of well ground nanoparticles was added, and the mixture was stirred for 10 min. After the addition of 100 μL of FITC-APTS conjugate, the solution was stirred for another 24 hr to complete the labeling. After the labeling,
nanoparticles were washed several times by nano-pure water and ethanol, and finally resuspended in water.

Fluorescence microscopic observation was performed at room temperature using a fluorescence microscope. A drop of the FITC-modified nanoparticle suspension was placed on a glass slide and used as a sample. Fluorescence and optical microscopic images were both taken and compared. The hydrophilic part of the nanoparticle surface was fluorescent. Confocal fluorescence microscope (Zeiss 780 confocal upright fixed stage confocal microscope, Carl Zeiss AG, Oberkochen, Germany) was also employed to observe the selective fluorescence labeled-nanoparticles.

![Figure 3.9: Procedures for labeling nanoparticles with APTS-FITC.](image)

Taken from Ikeda et al. 210.
The total area of fluorescent region on the particle surface was quantified by using ImageJ (developed by National Institutes of Health). A fluorescent ratio was calculated as the ratio of fluorescent area over total surface area, and was used as a measure of heterogeneity in surface hydrophobicity. The smaller the fluorescent ratio, the more hydrophobic the surface is.

### 3.6 Experimental approaches for the attachment of nanoparticles to bacterial surface

#### 3.6.1 Preparation of porous media

Spherical silicate glass beads (Potters Industries Inc., Berwyn, PA) were used as the porous medium, and were cleaned following a procedure established previously to remove extraneous materials initially present on the glass bead surface. The roughness on the glass bead surface was improved by stirring them in water with metal paddles of a stirring machine, so that a better adherence of biofilm could be achieved on a roughened glass bead surface. The glass beads (GB) were sterilized by autoclaving for 30 min before wet packed into a 10 cm-long glass column (C10/10, GE Healthcare, NJ), with an inner diameter of 10 mm and porosity of approximately 0.36.

Gram-negative *Pseudomonas aeruginosa* (PA) and Gram-positive *Bacillus cereus* (BC) were obtained from Dr. Claudia Gunsch Lab (Duke University, Durham NC). A single colony was transferred from a streaked plate into Lysogeny broth (LB), and incubated at 37°C in a shaking incubator for 20 h prior to inoculation. Then 100 mL of this preculture was mixed with 2 L of synthetic nutrient solution (composition of this
nutrient solution can be found in Table C1) and recirculated through a clear PVC column filled with polyurethane foam at 20 mL/min to fast grow biofilm for 2 days. After the formation of biofilm, 200 ml of this biofilm-containing recirculating solution was diluted in 2 L of synthetic nutrient solution and used to seed the GB column. The biofilm-containing solution was recirculated at a flow rate of approximately 1 mL/min and the flow direction was switched between upward and downward every 12 h to homogenize the spatial distribution of biofilm in the column. After attached to the surface of GB, the growth of biofilm stabilized after 3 days and the bacteria concentration in the effluent became stable (monitored by measuring the optical density (OD) at 600 nm). All the columns and tubing used in this experiment were sterilized by soaking them in bleach solution and then rinsing with autoclaved nano-pure water. Sterilizing-grade gas filters (Millipore, Billerica, MA) were employed to sterilize the air for aerating the synthetic nutrient solution.

To coat GB with BSA, the BSA solution (0.5 mg/mL, Thermo Fisher Scientific, Waltham, MA) was recirculated through the GB column for 1 day, as this concentration of BSA and contact time would ensure that the surface of GB was saturated by BSA \textsuperscript{309}. For alginate, the GB were pre-coated with a layer of cationic poly-L-lysine (PLL) following a protocol described elsewhere \textsuperscript{310}, in order for alginate to attach to the GB. Then the alginic acid sodium salt solution (2 g/L, from brown algae, Sigma-Aldrich, St. Louis, MO) was recirculated through the column filled with PLL-coated GB for 1 day.
3.6.2 Characterization of porous media

The spatial distribution of biofilm in the column was determined by measuring the total biomass in five separate segments. The column was divided into five 2 cm-long sections, and the total biomass in each section was calculated from the chemical oxygen demand (COD) measurements. After sonication and removing from GB, the oxidisable matter in biofilms, expressed by COD, was quantified with Hach COD test kit (Method 10067, Hach, Loveland, CO). The biomass then was then calculated by applying a conversion factor of 0.706 mg dry weight (DW) / mg COD.

The EPM and ζ-potential of GB were determined by Zeta Sizer Nano ZS (Malvern, Bedford, MA) using crushed GB. For porous media coated with biofilm, BSA or alginate, the electrokinetic property was characterized by measuring the EPM and ζ-potential of solutions containing biofilm, BSA or alginate. Hydrophobicity of collector surface was characterized by employing the organic dye adsorption method as described section 2.2.3. The same methods were also employed to characterize the EPM and hydrophobicity for planktonic bacteria.

After biofilm was removed from GB by sonication, EPS extraction from the biofilm was conducted in the presence of cation exchange resin (J. T. Baker, Mansfield, MA) following a protocol described elsewhere. The proteins content of EPS was quantified by the bicinchoninic acid (BCA) assay with BCA™ Protein Assay kit.
(Thermo Fisher Scientific, Waltham, MA) using BSA as standard; while he polysaccharides content was measured by DuBois method using glucose as standard.

### 3.6.3 Column experiments

The retention of nanoparticles at different collector surface was investigated by column experiments and the types of collector surface evaluated were: (1) clean GB, (2) gram-negative PA biofilm-coated GB, (3) gram-positive BC biofilm-coated GB, (4) BSA-coated GB, and (5) alginate-coated GB. The background electrolyte solution was 1 mM of NaCl, an environmentally relevant concentration of monovalent ions. None of the nanoparticles studied in this work was found to aggregate to an observable degree at this ionic strength. As soon as the biofilm was ready per aforementioned, at least 20 pore-volume of background electrolyte solution was pumped through the column (1 mL/min) to elute loose biofilm and residual impurities, and to equilibrate the column until the suspended solid concentration in the effluent was constantly low (as monitored by OD). Nanoparticle suspension was then introduced into the column at a flow rate of 0.05 mL/min for 5-6 pore volumes, followed by background electrolyte elution for another 10 pore volumes. The flow direction was upward. Samples from the effluent were collected intermittently at a rate of 0.5 mL/min and the nanoparticle concentrations were quantified afterward. The aqu-nC$_{60}$ concentration was measured by liquid-liquid extraction coupled with HPLC-UV/vis spectroscopy. UV/vis spectroscopy was used to quantify the concentration of fullerol. The concentrations of silver nanoparticles were
determined by ICP-MS with HNO$_3$/HCl pre-digestion as described elsewhere $^{305}$. To investigate the effect of divalent cations on the transport of nanoparticle in biofilm-coated porous media, 10 mM of CaCl$_2$ was flown through for 1h (approximately 20 pore volume) before the background electrolyte solution was applied and column experiments were carried out.

Upon measurement of the break through curves (BTC) for different nanoparticles, the attachment efficiency ($\alpha$) was calculated by Equation 3.4 $^2$.

**Equation 3.4:**

$$\alpha = -\frac{4r_c}{3(1-\varepsilon)\eta_0 L} \ln \left( \frac{C}{C_0} \right)$$

where $r_c$ is the geometric radius of collector, $\varepsilon$ and $L$ are the porosity and length of the porous medium, $C/C_0$ is the ratio of nanoparticle concentration in the effluent over that in the influent. $\eta_0$ is the single-collector efficiency, which is calculated by utilizing a correlation equation $^{316}$.

### 3.6.4 Preparation of planktonic bacteria for NPs attachment experiment

A single colony of PA or BC was transferred from a streaked plate into Lysogeny broth (LB), and incubated at 37°C in a shaking incubator for 20 h prior to nanoparticles attachment experiments. Then this preculture was washed three times with a buffer solution (10 mM NaCl, 4 mM NaHCO$_3$) and centrifuged at 1500g for 3 min. After washing, the pellet was re-suspended in the buffer solution for experiments.

The EPS-extracted bacteria were prepared using cation exchange resin (CER) $^{317}$, because the resin can be removed readily and it causes minimal cell lysis $^{221}$. The washed
bacteria pellet was suspended in an extraction buffer (2 mM Na$_3$PO$_4$, 4 mM NaH$_2$PO$_4$, 9 mM NaCl, 1 mM KCl, pH = 7) in 250-mL flasks centrifuge tubes, such that the concentration of bacteria was approximately $1 \times 10^8$ CFU/mL. 0.1 g/mL of CER was added and then the mixture was shaken on an orbital shaker for 30 min. After the end of shaking, the resin settled out of the solution. The solution of bacteria was then centrifuged at 1500g for 3 min and washed once with buffer solution. The pellet was re-suspended in the buffer solution for experiments as the EPS-extracted bacteria.

### 3.6.5 Batch attachment experiment

To perform the nanoparticles attachment experiments, pre-washed bacteria and EPS-extracted bacteria were spike to a series of nanoparticle suspension of different concentration to make the final concentration of bacteria $1 \times 10^8$ CFU/mL. The time needed for the attachment to reach equilibrium was determined beforehand by monitoring the change of unattached nanoparticle concentration over time. To conduct the batch attachment experiment, the mixture was shaken on an orbital shaker for 30 min at room temperature and then centrifuged at 1500g for 3 min. The concentration of nanoparticles in the supernatant was measured by UV/Vis spectroscopy and considered as the nanoparticles that were not associated with the bacteria, as the centrifugation force and time were optimized to minimize unattached nanoparticles in the pellet. Together with the concentration of nanoparticles before contact with bacteria, an adsorption isotherm was calculated. Control experiments were also conducted without
bacteria to account for the adsorbed nanoparticles to the glassware as well as the loss of nanoparticles in centrifugation due to possible aggregation, though no significant aggregation was observed after the nanoparticle suspension was mixed with the buffer solution. There was no growth of bacteria during the period of contact as confirmed by optical density measurement, and the cell lysis was also not significant as checked by LIVE/DEAD® viability assay (Invitrogen, Carlsbad, CA, USA).

The working absorption wavelength for the quantification of silver nanoparticles by UV/Vis spectroscopy was 405 nm, while it was 536 nm for gold nanoparticles. The UV/Vis absorbance at 347 nm and 400 nm was used to measure the concentration of fullerene and fullerol, respectively.

To investigate the effect of pH and IS on the interaction of nanoparticles with bacteria, the pH was adjusted by trace-metal grade HCl and NaOH; and different concentrations of NaCl were added to change the IS to the desired level. The pH range investigated was 5 – 9, which was considered as the environment relevant range for pH without causing bacterial lysis.
4 Characterizing hydrophobicity based on phase distribution: Theoretic basis and experimental study of the distribution of nanoparticles in a water-oil mixture and their behavior at the water-oil interface

4.1 Introduction

The increasing use of engineered nanomaterial in numerous industrial applications and consumer products is driving interest in evaluating potential risk these materials may pose to human health and ecosystems\textsuperscript{1, 17, 320}. A key step in this endeavor is to determine the parameters that are relevant to predicting, partitioning, degradation, transformation and bioamplification of pollutants\textsuperscript{12}. In comparison with organic compounds that have typically been the subject of risk evaluations over past half century, the particulate nature of many nanomaterials poses specific challenges in identifying the parameters to predict the physiological or environmental compartments where these materials are most likely to accumulate\textsuperscript{159}. For example, the octanol-water partition coefficient (K\textsubscript{ow}), has proven to be a useful indicator of the environmental concentration and exposure of organic compounds as applied to traditional risk assessment\textsuperscript{9} while the utility and interpretation of K\textsubscript{ow} for predicting nanoparticle exposure is still largely unknown\textsuperscript{27}. Brownian motion should be efficient in guiding nanoparticles to a preferred phase that minimizes the total free energy of the system\textsuperscript{117}. However, path-dependency of nanoparticle transport and the potential for nanoparticles to accumulate at the liquid-liquid interface casts some doubts on the direct extension to
nanoparticles of principles previously applied for organic compounds. A fundamental assumption in interpreting $K_{ow}$ is that molecules freely diffuse between the two liquid phases yielding an equilibrium distribution that reflects the relative affinity of the compound for each phase. The introduction of a third phase in the form of a nanoparticle may constrain diffusion at the liquid-liquid interface. Particle size and complexity of surface properties introduce additional challenges in interpreting the meaning of $K_{ow}$ for the case of nanoparticles.

The behavior, as well as the distribution, of nanoparticles in a two-phase immiscible liquid mixture, such as water-octanol, is also a topic of long term interest and of notable practical importance. The partitioning of nanoparticles into lipid-rich environments, such as the sea-surface microlayer, provides an important sink for nanoparticles. Studies that show carbon-based nanoparticles strongly partition into cellular compounds, such as lipids which may be hydrophobic relative to water, suggest that studying the distribution of nanoparticles in two immiscible liquids may provide useful information for nano-toxicity research. Molecular dynamics (MD) simulations have provided insight on this phenomenon, while only a limited number of experimental studies are reported.

Engineered nanoparticles are not only distinct from their molecular counterparts in presenting a third phase. They may have heterogeneous surfaces, including surface coatings composed of macromolecules, that combine with the properties of the
nanoparticle core to confound the interpretation of interfacial phenomena\textsuperscript{15-16}. One premise of the current work is therefore that the distribution and behavior of engineered nanoparticles in water-oil system are controlled by size, surface charge and surface treatments that may affect hydrophobicity.

In this study, the octanol-water pair was chosen as a reference for an immiscible liquid mixture and five different engineered nanoparticles were investigated for this system. Calculations based on the thermodynamics of particle disposition at the interface between these two liquids are compared with experiments.

4.2 Characteristics of nanoparticle suspensions

Five different engineered particles dispersed in nano-pure water were studied in this work: aqu-nC\textsubscript{60}, fullerol, Au-CIT, Ag-CIT, and Ag-PVP of two different sizes (small Ag-PVP and large Ag-PVP). The main characteristics were summarized in Table 4.1. The hydrophobicity of these nanoparticles was characterized by a variety of methods in Chapter 5 (Figure 5.8). It was found that aqu-nC\textsubscript{60} was the most hydrophobic, followed by Ag-PVP; and fullerol was the most hydrophilic, followed by Ag-CIT and Au-CIT. The surface of Ag-PVP was rendered amphiphilic due to the presence of surface coatings.

4.3 Thermodynamic considerations

We consider three compartments where nanoparticles may distribute in an immiscible water-oil system: the bulk phase of water, oil and the water-oil interface. If a particle is transferred from one of these compartments to the other, a change of free
energy occurs that can be calculated to determine whether or not such a transfer is thermodynamically favored \textsuperscript{117, 119, 131}.

Table 4.1: Characteristics of nanoparticle suspensions.

<table>
<thead>
<tr>
<th>NPs</th>
<th>Size\textsuperscript{a}, diameter/nm</th>
<th>Hydrodynamic diameter\textsuperscript{b}/nm</th>
<th>EPM/(\sqrt{\text{V}/s})</th>
<th>(\zeta)-potential/mV</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqu-nC(_{60})</td>
<td>85(\pm)38</td>
<td>91.9(\pm)25.5</td>
<td>-2.740(\pm)0.033</td>
<td>-34.97(\pm)0.42</td>
<td>Lab prepared</td>
</tr>
<tr>
<td>Fullerol</td>
<td>98(\pm)44</td>
<td>102.7(\pm)20.9</td>
<td>-4.560(\pm)0.027</td>
<td>-58.21(\pm)0.34</td>
<td>Lab prepared</td>
</tr>
<tr>
<td>Au-CIT</td>
<td>13(\pm)2</td>
<td>26.7(\pm)9.9</td>
<td>-2.920(\pm)0.022</td>
<td>-37.27(\pm)0.28</td>
<td>CEINT</td>
</tr>
<tr>
<td>Ag-CIT</td>
<td>17(\pm)3</td>
<td>24.8(\pm)11.6</td>
<td>-3.110(\pm)0.124</td>
<td>-39.70(\pm)1.58</td>
<td>CEINT</td>
</tr>
<tr>
<td>Ag-PVP (small)</td>
<td>8(\pm)2</td>
<td>32.6(\pm)17.4</td>
<td>-0.385(\pm)0.016</td>
<td>-4.91(\pm)0.21</td>
<td>CEINT</td>
</tr>
<tr>
<td>Ag-PVP (large)</td>
<td>39(\pm)10</td>
<td>44.7(\pm)11.6</td>
<td>-0.711(\pm)0.028</td>
<td>-9.07(\pm)0.36</td>
<td>CEINT</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Size determined by TEM image analysis, all values are means \(\pm\) SD;

\textsuperscript{b} The second order average hydrodynamic diameter given by DLS \(\pm\) standard deviation;

\textsuperscript{c} All values are means \(\pm\) 95\% confidence interval (n = 3);

\subsection*{4.3.1 Thermodynamic model involving solid-liquid interfacial energy}

Considering the interfacial energy between different phases, the change of free energy when moving a particle of radius R from water to oil is given by,

\textbf{Equation 4.1:} \(\Delta G = 4\pi R^2 (\gamma_{so} - \gamma_{sw})\)

where \(\gamma\) is the interfacial tension, and s, w and o denote solid, water and oil, respectively. Making use of Young’s equation,

\textbf{Equation 4.2:} \(\gamma_{so} - \gamma_{sw} = \gamma_{ow}\cos \theta\)

where \(\theta\) is the contact angle of the particle at the water-oil interface (Figure A1).

At equilibrium, since \(\Delta G\) is equal to \(-kT\ln D_{ow}\), it gives,
Equation 4.3: \( D_{ow} = \frac{[\text{Particle}]_{oil}}{[\text{Particle}]_{water}} = \exp \left( \frac{4\pi R^2 \gamma_{ow} \cos \theta}{-kT} \right) \)

The Maxwell-Boltzmann approach yields a similar expression for \( D_{ow} \). This equation suggests that, in addition to temperature, \( D_{ow} \) is controlled by particle size \( R \) and hydrophobicity (i.e. \( \theta \)). When \( \theta \) is less than 90°, indicating hydrophilic particle (Figure A1), \( D_{ow} \) is less than 1 corresponding to a particle with a higher affinity for water. This is consistent with the use of \( D_{ow} \) to characterize hydrophobicity. The larger the size, the further away \( D_{ow} \) is from 1 which suggests that the larger particles are more likely to stay at the thermodynamically favorable phase compared to smaller ones.

In addition, when a particle is removed from the water-oil interface into the water or oil, the solid-oil or solid-water interface is replaced by an incremental solid-water or solid-oil interface, and a new water-oil interface is created as a result. The free energy change of moving particle from the water-oil interface into water, for example, is given by,

Equation 4.4: \( \Delta G = 2\pi R^2 (1 - \cos \theta) \gamma_{sw} + \pi R^2 \sin^2 \theta \gamma_{ow} - 2\pi R^2 (1 - \cos \theta) \gamma_{so} \)

Again, making use of Young’s equation and it follows,

Equation 4.5: \( \Delta G = \pi R^2 \gamma_{ow} (1 - \cos \theta)^2 \)

By an analogous approach, the free energy change of moving a particle from the interface towards the oil phase is,

Equation 4.6: \( \Delta G = \pi R^2 \gamma_{ow} (1 + \cos \theta)^2 \)
Since here $\Delta G$ is always non-negative (Equation 4.5 and 4.6), it follows that an energy well exists for a partially wettable particle (i.e. $\theta \neq 0$ or $90^\circ$). This energy well is also controlled by particle size and hydrophobicity. Figure A2 shows the magnitude of the energy well ($\Delta G/kT$) for particles of different size and hydrophobicity at the water-octanol interface ($\gamma_{ow} = 8.52$ m N/m at $20^\circ$C). For a 1 nm particle, the energy wells are generally below one kT. The shallowness of the energy well in comparison with Brownian motion suggests that the particles in this case are not likely to be trapped at the interface. However, for a 10 nm particle, as the contact angle increase towards $90^\circ$, the energy well increases from below one $kT$ to values significantly larger than $kT$. It implies that the energy well of a larger amphiphilic (i.e. $\theta$ is close to $90^\circ$, Figure A2) particle becomes very deep. The energy well becomes overwhelmingly large for 100 nm particles suggesting that they can hardly escape from the interface.

For amphiphilic particles with contact angle $\theta = 90^\circ$ and a size as small as 1 nm, the energy well is less than $kT$; while it is larger than $kT$ for a 10 nm particle (Figure A3). Based on this calculation, the size 1~10 nm represents a threshold beyond which the particle will accumulate at the water-oil interface with no exception.

### 4.3.2 Thermodynamic model involving cavity formation energy

Although the interfacial energy between solid and liquid, $\gamma_{sl}$, is a convenient treatment to a rather complicated surface phenomena between a solute particle and the solvent, in practice $\gamma_{sw}$ and $\gamma_{so}$ are not accessible. Together with the application of
Young’s equation (Equation 4.2) and contact angle θ, γ_{sl} gives a simplified interpretation on the free energy of particle in solvent that overlooks many details.

Alternatively, the process of introducing a particle into a solvent can be considered to consist of two steps. A cavity of suitable size is first created to accommodate the particle, requiring a partial molar Gibbs free energy, \( \bar{\mathcal{G}}_c \). The particle introduced into this cavity then interacts with the solvent with a molar reversible work of, \( \bar{\mathcal{G}}_i \). In this manner the Gibbs free energy for a particle in a solvent, \( G \), is calculated as \( \frac{\sigma }{70} \).

**Equation 4.7: \( G = \bar{\mathcal{G}}_c + \bar{\mathcal{G}}_i + R T \ln (R T / 10^3) \)**

The cavity formation energy, \( \bar{\mathcal{G}}_c \), includes the reversible work needed to exclude solvent of volume \( V_p \) (volume of the particle) and the work needed to create a new interface between solvent and vapor under the surface tension of the liquid \( \gamma_i \). In other words, as \( \bar{\mathcal{G}}_c + \bar{\mathcal{G}}_i \) includes all the information of the interfacial tension \( \gamma_{sl}, \gamma_{li} \) can be considered as the sum of the surface tension of the liquid \( \gamma_i \) and the interaction between solid and liquid.

The cavity formation energy, \( \bar{\mathcal{G}}_c \), can be calculated according to scaled particle theory as \( \frac{\sigma }{70} \).

**Equation 4.8: \( \bar{\mathcal{G}}_c = -RT \ln (1 - y) + RT \frac{3y}{1-y} \left( \frac{\sigma_2}{\sigma_1} \right) + RT \left[ \frac{3y}{1-y} \frac{9}{2} \left( \frac{y}{1-y} \right)^2 \left( \frac{\sigma_2}{\sigma_1} \right) + \frac{N y P}{\rho} \left( \frac{\sigma_2}{\sigma_1} \right)^3 \right] \)**

where \( \sigma_1 \) and \( \sigma_2 \) are the molecular diameter of solvent and solute, respectively, \( q \) is the number density of solvent, and the compactness factor \( y = \pi \rho \sigma_1^2 / 6 \).
Table A1 lists some physical parameters of common organic solvents and calculations of $\tilde{G}_c$ for particles of different sizes. In the nano-range (i.e. 1 – 100 nm), $\tilde{G}_c$ in water is larger than that of any organic solvent (Table A1). Small size, as well as the high packing density, of the solvent molecules leads to a higher value of $\tilde{G}_c$. The packing density of water is lower than most organic solvents (Table A1) due to the hydrogen bonding, suggesting the smaller size of water molecule compared to that of other solvents is the reason for the higher $\tilde{G}_c$. Although debatable, it can be argued that this is the origin of the low solubility of hydrophobic molecules in water. Figure A4 shows the change of $\tilde{G}_c$, normalized by surface area, as size changes, and it is clear that $\tilde{G}_c$ grows linearly with surface area in the nano-range. This has also been suggested as an explanation for why hydrophobic particles self-assemble in water. Because the aggregation leads to the decreasing in specific surface area, it thus results in a lowered $G_c$.

As for $G_i$, without the presence of stronger interaction such as hydrogen bonding, van der Waals force serves as the major contributor. Therefore, the strong van der Waals attraction, or lack of, between particle and solvent can change $G_i$ to a degree that might or might not change the total solvation free energy, $G_i$ between a particle and solvent as given by,
Equation 4.9: \( \bar{G}_i = \frac{1}{24} A \left[ -\frac{(R^2-\sigma_{12}^2)(4R^3-3R^2\sigma_{12}^2+2R\sigma_{12}^2+\sigma_{12}^3)}{R^3\sigma_{12}^3} + 8 \ln \left( \frac{R}{\sigma_{12}} \right) \right] \)

where A is the Hamaker constant, R is particle radius, and \( \sigma_{12} = \frac{\sigma_1 + \sigma_2}{2} \).

Derivation of Equation 4.9 can be found in Appendix B.

Using Equation 4.9, \( \bar{G}_i \) for different engineered nanoparticles in both water and octanol was calculated (Table A2). The interaction energy between particles and octanol were smaller compared to that between particles and water, with larger molecular size of octanol being the possible reason. As same as \( \bar{G}_c \), \( \bar{G}_i \) also changed proportional to surface area as shown in Equation 4.9. Thus, \( G_r \) as well as \( \Delta G \), was also expected to be linear with surface area, which was consistent with the prediction made previously. Calculations of the \( \Delta G \) for moving particle from water to oil (Equation 4.10) are also summarized in Table A2.

Equation 4.10: \( \Delta G = G_o - G_w = (\bar{G}_c + \bar{G}_i)_o - (\bar{G}_c + \bar{G}_i)_w \)

\( \Delta G \) for all of the nanoparticles calculated were negative, which indicated that they were all hydrophobic considering van der Waals interaction only. Another interesting interpretation from this result was that the origin of hydrophobicity might come from the high surface tension of water (i.e. the large cavity formation energy), rather than a low affinity for water. In fact, \( \bar{G}_i \) of all three nanoparticles in water was even larger than in octanol. However, in reality, some nanoparticles were observed to be quite hydrophilic, suggesting that some other interactions such as hydrogen bonds or charge-dipole interaction also played a role.
For solutes, either small molecules or particles, that can form hydrogen bonding with water molecule, the attraction between solute and solvent becomes significantly larger than the van der Waals force alone. Thus, the interaction energy should also include the free energy of hydrogen bonding which cannot be described straightforwardly.

The surface of nanoparticles dispersed in water is often charged, and the interaction between a charged atom and a polar molecule like water cannot be neglected. The ion-dipole interaction is strong enough to align water molecule, which may be treated as a simple spherical molecule of radius 0.14 nm with a point dipole of moment \(1.85 \text{D}\), or other polar molecules around charged particles. The interaction energy for ion-dipole interaction is a function of the valency of ion, dipole moment, and the distance between them. For example, the maximum interaction energy between \(\text{Na}^+\) and water is \(9.6 \times 10^4 \text{ J/mol}\) at 300 K. It is relatively smaller compared to \(\tilde{G}_c\) of a 10 nm particle in water (\(\tilde{G}_c = 1.05 \times 10^7 \text{ J/mol}\)). However, depending on the charge density on the particle surface, the ion-dipole interaction energy could be on par with \(\tilde{G}_c\) or even larger. Taking a 100 nm particle with a surface charge (monovalent ions) density of 0.005 C/m\(^2\) as an example, the ion-dipole interaction energy becomes \(9.42 \times 10^7 \text{ J/mol}\), which is much greater than \(\tilde{G}_c\) of a 10 nm particle in water.
Hydrogen bonds and ion-dipole interaction are two interactions that are, in some cases, stronger than van der Waals interaction and make hydrophobic particles hydrophilic or hydrophilic particle even more stable in water.

4.3.3 Interactions making particles less stable at the liquid-liquid interface

The theory discussed in previous sections suggests that particles larger than approximately 10 nm in diameter, independent of the hydrophobic or hydrophilic nature of their surfaces, are more likely to accumulate at the water-oil interface when the equilibrium is reached due to the presence of a deep energy well. However, some experimental results have suggested otherwise, and our own data also run contrary to this prediction. Therefore, the extremely large energy well at the interface must be neutralized by some other phenomenon that gives rise to positive energies in the interaction between particles and the interface. Possible candidates for such particle-particle repulsive interactions include electric double layer and steric interactions, as the surface of engineered nanoparticles is often charged and with polymeric coatings. For instance, charged nano-gold particles stabilized with citrate were found not to accumulate at the water-heptane interface; while when protonated by lowering pH, they did accumulate at the interface. Since the interaction between charged atoms with a polar solvent like water is greater than that with nonpolar molecule, charged particles may be treated as a special kind of “hydrophilic” particle. Thus a particle cannot be considered as an amphiphilic particle unless it is de-charged. In this case, it is consistent
with our hypothesis that only large amphiphilic particles would be trapped by the deep energy well at the interface.

4.4 Experimental results and discussions

4.4.1 Effect of pH on the distribution of nanoparticles in water-octanol system

We examined the effect of pH on the distribution of nanoparticle in water-octanol system in the range of pH values between 1.5 and 11.5. The distribution coefficient $D_{ow}$ of nanoparticles under different pH was shown in Figure 4.1. Three trends were observed in the distribution behavior of nanoparticles.

![Figure 4.1: $D_{ow}$ of different nanoparticles at different pH. Error bars represent ±1 standard deviation.](image_url)
For aqu-nC₆₀, it was either negatively charged in a high pH environment or positively charged when pH was low. D_{ow} of aqu-nC₆₀ gradually increased as pH approached an isoelectric point (IEP) between 1 and 3, though different IEP for aqu-nC₆₀ have been reported. But for higher pH values, the drop of D_{ow} was similar to the decrease in surface charge. Pristine fullerene, as a highly hydrophobic molecule and with a very low solubility in water (7.96 ng/l), was expected to overwhelmingly partition into a nonpolar solvent like octanol rather than water. But the surface of aqu-nC₆₀ was partially hydroxylated in the extended mixing process and thus, it was negatively charged at neutral pH (Table 4.1). The charged surface of aqu-nC₆₀ significantly increased its affinity with water as discussed previously. Therefore, the D_{ow} of aqu-nC₆₀ (D_{ow} = 3.08 at pH=7) was much smaller than that of pristine fullerene (D_{ow} = 106.67), and the lower surface charge around IEP led to an enhanced D_{ow} (D_{ow} = 6.32 at pH=3). The hydrodynamic size of aqu-nC₆₀ also increased, as pH got closer to the IEP, consistent with destabilization due to a more neutral surface. The percentage of nanoparticles at interface remained less than 10 %, and it was highest (9.82%) at pH = 3.69. Most of aqu-nC₆₀ aggregates partitioned into octanol, rather than accumulating at the interface, as a result of the relatively strong hydrophobicity of aqu-nC₆₀.

Fullerol, the hydroxylated derivative of fullerene, showed a completely different behavior. When pH was around its IEP (~3.94), surface charge was minimized and fullerol nanoparticles aggregated. However, D_{ow} was well below 0.15 with no statistical
difference over the entire range of pH, and less than 2% of fullerol nanoparticles were at
the interface. The 24 hydroxyl groups on fullerol surface, which could form hydrogen
bonds with water molecule, made it very hydrophilic. Thus it is thermodynamically
favorable for fullerol nanoparticles to stay in water compared to the interface or octanol,
even though surface charge diminished and size increased.

Compared to these two carbon-based nanoparticles, the behavior of nano-Ag and
nano-Au particles as pH changed was more nuanced. The changes of surface charge and
particle size for Au-CIT and Ag-CIT were similar with aqu-nC60 and fullerol; the
maximum of D_{ow} did not show up around their IEPs nonetheless. In contrast, the D_{ow}
dropped quickly after the solution became acidic. The dissolution of silver and gold
nanoparticles in the presence of acid might be responsible for the fact that majority of Ag
and Au \(_{96,325}\), in the form of ions, was in aqueous phase. Similar trend followed for Ag-
PVP, and it was also most likely due to dissolution. Since dissolution alters both the
overall measured distribution of Ag and the actual size of the Ag NPs, the
thermodynamic model discussed previously is not valid to interpret the experimental
results. Interestingly it was found that for the large Ag-PVP nanoparticle in a non-acid
environment, more than 38% were at the interface; while less than 8% of the small Ag-
PVP nanoparticles accumulated at the interface. Considering the low \(\zeta\)-potential of Ag-
PVP (-4.91 mV for smaller particles and -9.07 mV for large ones), the amphiphilic PVP
coatings might produce a more amphiphilic Ag-PVP surface thus making these particles
stable at the interface. However, the energy well for small Ag-PVP (< 10 nm) was not large enough to keep them at the interface as already discussed in the thermodynamic models.

**4.4.2 Effect of ionic strength on the distribution of nanoparticles in water-octanol system**

10 – 100 mM of NaNO₃ were added in order to change the surface charge without inducing dissolution as occurred with a change in pH. As the IS rose, the surface charges of aqu-nC₆₀, fullerol, Ag-CIT and Au-CIT decreased accordingly and their sizes constantly increased due to aggregation. The majority of carbon-based nanoparticle did not aggregate at the interface (12% of aqu-nC₆₀ and 4.5% of fullerol respectively). More aqu-nC₆₀, as illustrated by the larger D_{ow}, with less surface charge and larger size partition into octanol; while aggregated fullerol still remained in the water and D_{ow} was close to constant (Figure 4.2). Researchers have been taking advantage of this property of fullerene by adding salts to promote the extraction of fullerene from aqueous sample to organic solvent so as to quantify the concentration of fullerene 315.

For Ag-CIT and Au-CIT, as IS increased, the fraction of nanoparticles at the interface became significantly larger (Figure 4.3a and 4.3b). Calculated by difference, 3.8% of Ag-CIT and 7.9% of Au-CIT was at the interface when IS = 10mM and the size of neither increased by more than 10%. At IS = 50 mM, 27.6% of Ag-CIT and 34% of Au-CIT moved to the interface and their sizes were larger than 100 nm. Compared with 50 mM
IS, the distributions of Ag-CIT and Au-CIT at IS = 100 mM were similar. In addition, at higher IS, a large fraction of Ag-CIT and Au-CIT still remained in water. It indicated the relative hydrophilic property of them even when surface charge was almost neutralized.

![Graph](image)

**Figure 4.2: D_{ow} of aqu-nC60 and fullerol at different IS (in [NaCl]).** Error bars represent ±1 standard deviation.

The changing IS had minimal effect on the surface charge as well as particle size of Ag-PVP. As suggested by other studies, Ag-PVP nanoparticles are sterically stabilized by PVP polymer thus explaining the negligible effect of IS for these particles. Large Ag-PVP consistently congregated at the interface (38.7% - 39.2%, Figure 4.3d) at any IS, while smaller Ag-PVP mainly partitioned between water and octanol as discussed previously (Figure 4.3c). More Ag-PVP was in the octanol phase than water phase, which suggested that they were slightly hydrophobic.
Figure 4.3: Distribution of different nanoparticles between water, octanol and the water-octanol interface at different IS.
(a) Ag-CIT; (b) Au-CIT; (c) small Ag-PVP; (d) large Ag-PVP.

4.4.3 Effect of particle size on the distribution of nanoparticles in water-octanol system

The distribution of aqu-nC₆₀ suspensions with different mean sizes prepared by successive filtration was evaluated in water-octanol mixture at pH = 7, and the $D_{ow}$ data are shown in Figure 4.4. The majority of fullerene was found to partition into two bulk phases, rather than the interface. From Figure 4.4, $D_{ow}$ of aqu-nC₆₀ decreased as the size
got smaller, with $D_{ow}$ always being larger than 1. This again confirms that aqu-nC$_{60}$ was hydrophobic, thus favoring the octanol phase. But larger fullerene aggregates particles tended to move to the octanol, as predicted by the thermodynamic model. The calculated $\zeta$-potentials of the different fractions were similar (in the range of -28.9 to -39.1 mV), though the enhanced hydroxylation on the surface of smaller fullerene compared to larger ones might also contributed to the lowered $D_{ow}$ as hydroxyl groups were generally considered to be hydrophilic.

![Graph showing $D_{ow}$ of different size fractions of aqu-nC$_{60}$ at pH = 7](image)

**Figure 4.4: $D_{ow}$ of aqu-nC$_{60}$ of different size at pH = 7.**
Error bars represent ±1 standard deviation.

The different distribution of Au-CIT nanoparticles as their size changed was another example of the effect of particle size. As discussed earlier, by increasing the IS, Au-CIT became larger. Figure 4.5 showed the different distribution of Au-CIT at 10 mM and 50 mM of IS observed by the dark field microscope. Particle size at IS = 10 mM was ~10 nm, the gold nanoparticles were evenly distributed in the water phase and few of
them crossed the interface getting into the octanol (Figure 4.5a). However, at IS = 50 mM, the particle size increased to ~100 nm. Almost all of the particles accumulated at the interface, with only a few nanoparticles in octanol phase (Figure 4.5b). Similar effect of size on the distribution of nano-Au particle in a poly(ethylene glycol)-dextran system have been observed by Helfrich et al. 327.

![Figure 4.5: Distribution of Au-CIT with different size at different IS observed by dark field microscopy.](image)

(a) Size of Au-CIT = ~10 nm at IS = 10 mM; (b) Size of Au-CIT = ~100 nm at IS = 50 mM.

### 4.4.4 Path-dependent distribution of nanoparticles in water-octanol system

Previous experiments were all conducted by starting with nanoparticles in the aqueous phase and then distributed between water, octanol and the interface. After the equilibrium was reached (pH = 7), the octanol phase was mixed with fresh nano-pure water. The distribution of nanoparticle in this new system was measured (Figure 4.6). Similar results were found for aqu-nC60 and small Ag-PVP nanoparticle. In the new system where the starting point for nanoparticles distribution was octanol, the fraction
of particles in the water decreased greatly. For aqu-nC_{60}, it dropped from 24.2% to 8.7%; while for small Ag-PVP, it was from 28.3% to 14.5%. One possible explanation for this phenomenon was that when the direction of distribution was from water to octanol, those nanoparticles with less or no surface charge were those eventually ending up in the octanol phase due to a lower affinity for water. Thus, when those nanoparticles were mixed with fresh water, they tended to stay in the octanol. The calculated ζ-potentials of aqu-nC_{60} and Ag-PVP in the water phase of the second water-octanol mixture were smaller compared to those of the original solution, which confirms this hypothesis.

![Image of distribution](image)

**Figure 4.6**: Distribution of aqu-nC_{60} and small Ag-PVP with water or octanol as the starting point. (a) aqu-nC_{60}; and (b) small Ag-PVP.

### 4.5 Summary of results

Our overall results have shown that unlike small molecules like organic compounds, the distribution of nanoparticles in a water-oil like system is not solely
defined by the hydrophobicity, but also largely controlled by the surface charge and
particle size. While nanoparticles may not move around freely in the system and end up
in either bulk phase due to the existence of an energy trap at the water-oil interface, they
do not automatically congregate there either. Although there is no magic number for the
size beyond which the particle becomes “particulate” and will be trapped at the
interface; particles approximately 10 to 100 nm in size (i.e., nanoparticles by many
definitions) will to some degree accumulate at the interface depending on how
amphiphilic they are. Therefore, nanoparticles are, indeed, different from organic
compounds in terms of their behavior in such system. The traditional risk assessment
methods based on $K_{ow}$ that are useful for organic compounds need major adaption in
order to be applied in the world of nanoparticles.
5 Adsorption of molecular probes and a comparison with other methods for characterizing nanoparticle surface hydrophobicity


5.1 Introduction

As described in the previous chapter, the theoretical basis for partitioning methods at the molecular level is ambiguous, requiring at a minimum several caveats in interpreting results produced by methods similar to K_{ow} determination. In addition, surface coatings on nanoparticles, designed to accommodate the use of engineered NPs for their intended applications, may introduce a degree of heterogeneity to the surface properties of nanoparticles such as hydrophobicity. A heterogeneous surface, together with the properties of the nanoparticle core, makes the interpretation of interfacial phenomena complicated.

Current characterization methods for hydrophobicity can be summarized as falling into three categories: macro-scale, solute-scale and nano-scale. Measurements of contact angle are frequently used to obtain estimates of surface tension, employing the sessile drop Young–Laplace method. Applied under ideal conditions, the tested substance is a macroscopic, flat solid surface, thus requiring modification to apply to nanoparticles, for example, a thin film of nanoparticles. Several methods have been
proposed for measuring the contact angle of nanoparticles; however, most of them are indirect measurement by fitting thermodynamic or optical model to the experimental data and calculating contact angle. For the solute-scale partitioning method, the distribution of tested substance between two immiscible liquid phases, typically water and one organic solvent such as octanol, is measured. $K_{ow}$ for various organic compounds are widely reported in the literature. Adsorptive method evaluates the relative affinity of tested substances to a standard hydrophobic material. This latter method is potentially the most suitable for nano-scale measurements as it can be easily applied to materials of all sizes, and it takes advantage of an increased sensitivity associated with the high specific surface area of the nanomaterial to be tested.

There is another group of characterization methods that apply to particle powders and measure the affinity of water molecules for the surface of powders, such as the dynamic water vapor adsorption, immersion microcalorimetry and thermogravimetric analysis. Though these water-affinity based methods provide information about the interaction between a solid surface and water and are often used to infer hydrophobicity, the interpretation of the results is not the same as hydrophobicity, as in most of these methods the bulk water is not involved. Therefore, it is unclear that if a thermodynamic interpretation of the results can be obtained from water-affinity based characterization techniques.
In this study, macro-scale (i.e. contact angle measurement), solute-scale (i.e. partitioning coefficient measurement) and nano-scale (i.e. adsorptive method) characterization methods were employed to quantify the relative hydrophobicity of different nanoparticle suspensions. Several water-affinity based methods were also used to measure the hydrophobicity of nanomaterial powders. A comparison of different techniques was carried out with the intent to identify the best characterization method for hydrophobicity of nanoparticles.

5.2 Characteristics of nanoparticles suspensions

Seven different nanoparticles suspensions were characterized. The main characteristics of these NPs suspensions are presented in Table 5.1.

<table>
<thead>
<tr>
<th>NPs</th>
<th>Size(^{a})(nm)</th>
<th>EPM(^{b}) ((10^{-8} \text{m}^2/\text{V} \text{s}))</th>
<th>Concentration(^{c}) (mg/L)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqu-nC(_{60})</td>
<td>87 ± 11</td>
<td>-0.38 ± 0.02</td>
<td>22</td>
<td>Lab prepared(^{d})</td>
</tr>
<tr>
<td>THF-nC(_{60})</td>
<td>55 ± 12</td>
<td>-0.71 ± 0.03</td>
<td>14</td>
<td>Lab prepared</td>
</tr>
<tr>
<td>Fullerol</td>
<td>98 ± 7</td>
<td>-1.57 ± 0.03</td>
<td>80</td>
<td>Lab prepared</td>
</tr>
<tr>
<td>Au-CIT</td>
<td>31 ± 6</td>
<td>-2.60 ± 0.05</td>
<td>60</td>
<td>CEINT</td>
</tr>
<tr>
<td>Ag-CIT</td>
<td>52 ± 10</td>
<td>-3.12 ± 0.03</td>
<td>100</td>
<td>CEINT</td>
</tr>
<tr>
<td>Ag-PVP</td>
<td>48 ± 12</td>
<td>-1.21 ± 0.05</td>
<td>250</td>
<td>CEINT</td>
</tr>
<tr>
<td>Ag-GA</td>
<td>16 ± 7</td>
<td>-0.41 ± 0.03</td>
<td>250</td>
<td>CEINT</td>
</tr>
</tbody>
</table>

\(^{a}\) The second order average hydrodynamic diameter given by DLS ± standard deviation;

\(^{b}\) EPM showed in this table is mean ± 95% C.L.

\(^{c}\) Concentration of NPs in stock solutions;

\(^{d}\) Preparation methods refer to previous discussion in section 2.2.1
5.3 Macro-scale characterization method – the contact angle measurement on thin film of nanoparticles

Contact angle measurement results are shown in Table 5.2. Theoretically, a contact angle greater than 90° suggests that the tested surface is hydrophobic. Surprisingly, all the NPs yielded contact angles of less than 90°, though contact angles of aqu-nC₆₀, THF-nC₆₀, Ag-PVP and Ag-GA were close to 90°. Aqu-nC₆₀ and THF-nC₆₀ were the most hydrophobic, followed by Ag NPs and Au NPs. Fullerol was the most hydrophilic, as demonstrated by the smallest contact angle. The contact angles of aqu-nC₆₀ and THF-nC₆₀ showed in Table 5.2 were similar with that of graphite, 98.3°. The contact angle of water on gold and silver metal surface were reported to be 65° and 79°. The presence of citrate made both gold and silver surface more hydrophilic, while polymeric coatings had less impact.

Table 5.2: Contact angle on thin film of nanoparticles.

<table>
<thead>
<tr>
<th>NP</th>
<th>Aqu-nC₆₀ (Mean±95% C.L.)</th>
<th>THF-nC₆₀ (Mean±95% C.L.)</th>
<th>Fullerol (Mean±95% C.L.)</th>
<th>Au-CIT (Mean±95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Angle/°</td>
<td>82.6±3.8</td>
<td>81.5±3.4</td>
<td>35.7±2.1</td>
<td>41.2±2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NP</th>
<th>Ag-CIT (Mean±95% C.L.)</th>
<th>Ag-PVP (Mean±95% C.L.)</th>
<th>Ag-GA (Mean±95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Angle/°</td>
<td>40.4±1.8</td>
<td>78.7±2.9</td>
<td>78.9±3.2</td>
</tr>
</tbody>
</table>

There were no statistical differences between contact angles obtained for aqu-nC₆₀ and THF-nC₆₀, or Ag-PVP and Ag-GA. The contact angle is an average measure of the surface hydrophobicity and is useful in generally comparing hydrophobicity of
different NPs. However, it cannot give any detail about the heterogeneity in hydrophobicity on the surface due to the use of coatings.

5.4 Solute-scale characterization method – $K_{ow}$ measurement

$K_{ow}$ values greater than 1 indicate a hydrophobic material, while values less than 1 indicate a preference for the aqueous phase (Table 5.3). NPs determined to be more hydrophobic in the contact angle experiment yielded larger $K_{ow}$ values. $K_{ow}$ of NPs with PVP or GA coatings were close to 1, suggesting amphiphilic behavior that might have contributed to the emulsion zone between water and octanol in the experiments, with the coatings acting as surfactants. Citrate, PVP and GA were all water-soluble. However, $K_{ow}$ of both Ag-PVP and Ag-GA were larger than 1, while Ag-CIT had a $K_{ow}$ of less than 1. Others have reported a $K_{ow}$ of pristine fullerene to be 106.67\textsuperscript{324}, which is several order of magnitude larger than the value of $K_{ow}$ reported here and likely highlights the role of important role of kinetics as well as the effect of sonication on the surface property of fullerene when preparing aqu-nC\textsubscript{60} suspension. The $K_{ow}$ of THF-nC\textsubscript{60} was 2.54, falling between $K_{ow}$ of aqu-nC\textsubscript{60} reported here and the $K_{ow}$ of 1.66 reported for THF\textsuperscript{331}. The lower $K_{ow}$ value for THF-nC\textsubscript{60} is consistent with the hypothesis that small residual amounts of THF may have explained the reduced hydrophobicity of the THF-nC\textsubscript{60} compared to that of aqu-nC\textsubscript{60}. 

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Table 5.3: $K_{ow}$ of nanoparticles.

<table>
<thead>
<tr>
<th>NP</th>
<th>Aqu-nC₆₀</th>
<th>THF-nC₆₀</th>
<th>Fullerol</th>
<th>Au-CIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{ow}$</td>
<td>3.08±0.18</td>
<td>2.54±0.27</td>
<td>0.12±0.03</td>
<td>0.38±0.07</td>
</tr>
<tr>
<td>(Mean±95% C.L.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NP</th>
<th>Ag-CIT</th>
<th>Ag-PVP</th>
<th>Ag-GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{ow}$</td>
<td>0.03±0.009</td>
<td>2.26±0.17</td>
<td>2.14±0.34</td>
</tr>
<tr>
<td>(Mean±95% C.L.)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.5 **Nano-scale adsorptive method – in-situ adsorption of molecular probes**

5.5.1 **Organic dye adsorption on NPs in aqueous suspension**

Out of seven different NPs tested, RB adsorbed to five (Figure 5.1): Ag-PVP, Ag-GA, Au-CIT, aqu-nC₆₀, and THF-nC₆₀. However, there was no observable adsorption of RB on Ag-CIT or fullerol. Larger slopes in Figure 5.1 indicate more adsorption of RB per unit area of the NP surface, which implies that the NP surface was more hydrophobic.

Results in Figure 5.1 showed that by this measure, aqu-nC₆₀ was the most hydrophobic, having the highest slope, followed by THF-nC₆₀, Ag-PVP, Ag-GA and Au-CIT. Pristine fullerene is highly hydrophobic, as evidenced by its very low solubility in water (7.96 ng/L) [324]. The extended mixing when preparing aqu-nC₆₀ leads to partial hydroxylation of fullerene surface [306], hence making it adequately hydrophilic to be dispersed as a stable suspension in water, yet hydrophobic enough that they easily aggregate. The comparatively lower hydrophobicity of the THF-nC₆₀ may be due to the retention of residual THF on the surface of fullerene, which may form hydrogen bond with water to make THF-nC₆₀ more hydrophilic than aqu-nC₆₀ [289]. The coatings of citrate,
PVP or GA were meant to stabilize nano-Ag by steric shielding, but our results showed that coatings also changed the surface chemistry of nano-Ag. Ag-CIT was more hydrophilic compared to nano-Ag with PVP or GA as RB hardly adsorbed onto the Ag-CIT surface. PVP and GA made nano-Ag more hydrophobic as a result of the amphiphilic properties of PVP and GA.

![Graph showing relative hydrophobicity](image)

**Figure 5.1:** Relative hydrophobicity as measured by adsorption of RB on nanoparticles surface. Error bars represent ±1 standard deviation.

The limitation of RB adsorption was that it was not applicable to particle surfaces that were more hydrophilic than carboxylated or hydroxylated polystyrene particles as demonstrated by the failure of RB to adsorb on such surfaces. Thus, as shown in our study, Ag-CIT and fullerol were quite more hydrophilic, consistent with the presence of carboxyl groups in the citrate and the hydroxyl groups on the fullerol. The relative
The hydrophobicity of fullerol and Ag-CIT can be interpreted from the adsorption of a hydrophilic dye, Nile Blue (NB). Following the same procedure as RB adsorption experiment with NB, the results were shown in Figure 5.2, and the larger slopes in this figure indicate less hydrophobic particle. By this measure, fullerol was much more hydrophilic than either the Ag-CIT or Au-CIT. Compared with aqu-nC₆₀ and THF-nC₆₀, the 24 hydroxyl groups in fullerol made it more hydrophilic and hence an enhanced solubility in water. Interestingly, little difference in hydrophobicity between Ag-CIT and Au-CIT was observed despite the differences in core material. These results demonstrate that the surface hydrophobicity of coated NPs largely depended on the properties of coatings, rather than the properties of the core materials.

![Figure 5.2: Relative hydrophobicity as measured by adsorption of Nile Blue on nanoparticles surface.](image)

Error bars represent ±1 standard deviation.
Another limitation of using organic dyes adsorption to characterize surface hydrophobicity was the interference of electrostatic interaction. RB was negatively charged under the experimental condition, while NB was positively charged. According to the EPM measurements shown in Table 5.1, all the NPs investigated in this study were negatively charged. Thus the repulsive or attractive forces between RB or NB and NPs might reduce or enhance the adsorption of dyes on NPs. Although some studies such as Gessner et al.\textsuperscript{333} reported that similar amount of RB adsorbed on particles of same material and significantly different surface charge which suggested that surface hydrophobicity might have a larger role in the adsorption, the importance of electrostatic interaction cannot be underestimated. Therefore a neutral hydrophobic molecule such as naphthalene might be a better probe than charged organic dyes.

5.5.2 Naphthalene adsorption on NPs in aqueous suspension

Experimental results for the adsorption of naphthalene on different NPs are summarized in Table 5.4 and Figure 5.3. The adsorption data were fitted to the Freundlich isotherm (Equation 5.1).

\textbf{Equation 5.1:} \( q = K_f C_w^{1/n} \)

where \( q \) (mg/g) is the mass of naphthalene per unit mass of NPs, \( K_f \) (L\(^{1-1/n}\) mg\(^{1-1/n}\) g\(^{-1}\)) is the Freundlich constant, \( C_w \) (mg/L) is the equilibrium concentration of naphthalene in aqueous phase and \( 1/n \) is the Freundlich exponent.
Figure 5.3: Adsorption isotherm of naphthalene on nanoparticles surface. Error bars represent ±1 standard deviation.

Table 5.4: Freundlich isotherm parameters for adsorption of naphthalene to nanoparticles.

<table>
<thead>
<tr>
<th>NP</th>
<th>$K_F$ ($L^{1/n} \cdot mg^{1-1/n}/g$)</th>
<th>$N$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqu-nC₆₀</td>
<td>$13.16 \pm 0.24$</td>
<td>$2.24 \pm 0.10$</td>
<td>0.997</td>
</tr>
<tr>
<td>THF-nC₆₀</td>
<td>$12.64 \pm 0.25$</td>
<td>$2.10 \pm 0.07$</td>
<td>0.993</td>
</tr>
<tr>
<td>Fullerol</td>
<td>$15.56 \pm 0.25$</td>
<td>$2.16 \pm 0.01$</td>
<td>0.999</td>
</tr>
<tr>
<td>Ag-PVP</td>
<td>$10.33 \pm 0.12$</td>
<td>$1.56 \pm 0.09$</td>
<td>0.996</td>
</tr>
<tr>
<td>Ag-GA</td>
<td>$9.99 \pm 0.28$</td>
<td>$1.55 \pm 0.02$</td>
<td>0.993</td>
</tr>
</tbody>
</table>

$^a$ Freundlich constant in this table is mean ± 95% C.L.

$^b$ $N$ in this table is mean ± 95% C.L.

$R^2$ results in Table 5.4 (> 0.99) suggested that the Freundlich isotherm was a reasonable choice for the adsorption of naphthalene on NPs. No observable adsorption on Ag-CIT and Au-CIT occurred during the experiments, which was consistent with the hydrophilic nature of these two NPs as determined by the previous measures. Comparing with the results of organic dye adsorption experiments, an obvious
difference was that more naphthalene adsorbed to fullerol than aqu-nC₆₀, which
contradicts the common expectation that fullerol is more hydrophilic than aqu-nC\textsubscript{60}. Lin et al \textsuperscript{334} and Chen et al \textsuperscript{174} proposed a possible explanation to this observation; the adsorption of adsorbates with aromatic rings on carbon-based NPs such as fullerene and carbon nanotubes (CNT) is not solely controlled by hydrophobic interaction, but also by $\pi-\pi$ interaction. Fullerene and naphthalene are both $\pi$-electron rich molecules. The hydroxyl groups of fullerol, acting as electron-donating substituent on fullerene surface, can strengthen the $\pi-\pi$ interaction hence enhancing the adsorption of naphthalene on fullerol to a larger extent than on fullerene. Except for fullerol, the results agreed well with the organic dye adsorption experiments, in that aqu-nC\textsubscript{60} was the most hydrophobic with more naphthalene adsorption followed by THF-nC\textsubscript{60}, Ag-PVP and Ag-GA.

We note that when characterizing surface hydrophobicity by adsorption, special attention needs to be paid to ruling out other mechanisms that might be involved in the adsorption process, such as electrostatic attraction and other chemical bonding like $\pi-\pi$ interaction in this case.

### 5.6 Water-affinity based characterization methods applied to nanomaterial powders

#### 5.6.1 Thermodynamic consideration

The film pressure $\pi_S$, given by Equation 5.2, describes the decrease of free energy accompanying the adsorption of vapor on a solid surface, at constant temperature.
Equation 5.2: $\pi_S = \gamma_S - \gamma_{SV}$

where $\gamma_S$ is the surface energy of solid and $\gamma_{SV}$ is the solid-vapor interfacial energy. $\pi_S$ is a function of pressure, and can be calculated using Gibbs adsorption equation (Equation 5.3)\(^{35}\),

Equation 5.3: $\pi_S = RT \int \Gamma d \ln P$

where $\Gamma$ is the Gibbs surface excess of the adsorbate. The equilibrium film pressure, $\pi_e$, can be obtained from Equation 5.3 by integration between 0 and $P_0$, using the vapor adsorption isotherms. Moreover, $\pi_e$ is equal to the negative free energy change of vapor adsorption process, $-\Delta_{ads} G$.

For immersion calorimetry experiments, the particle powders under investigation can be partially, fully or not at all covered by the vapor of the immersion liquid. When the particle powders, which have no vapor molecules on the surface, are immersed into the liquid, the following equation applies,

Equation 5.4: $\Delta_{imm} G = \gamma_{SL} - \gamma_S$

Using Young’s equation and Equation 5.2, Equation 5.4 becomes,

Equation 5.5: $\Delta_{imm} G = -\pi_e - \gamma_L \cos \theta$

where $\gamma_L$ is the surface energy of the liquid, and $\theta$ is the contact angle of the water drop formed at the solid surface. Thus, applying the definition of the Gibb’s free energy,
Equation 5.6: \( \Delta_{imm}H = \Delta_{imm}G + T\Delta_{imm}S = -\pi_e - \gamma_L \cos \theta - KT \)

where \( K = \frac{dy_{SL}}{dT} - \frac{dy_S}{dT} \), and it can be calculated by assuming the temperature dependence of solid surface energy and solid-liquid interfacial energy as \(-7 \times 10^{-5} \text{J/m}^2\) \( K \) and \( 0 \) \(^{335} \), respectively. Rearrange Equation 5.6, it yields,

Equation 5.7: \( \cos \theta = \frac{-KT - \Delta_{imm}H - \pi_e}{\gamma_L} \)

Equation 5.7 indicates that the contact angle of particle powders can be obtained by measuring the immersion enthalpy and equilibrium film pressure from immersion and vapor adsorption experiment, respectively \(^{350} \).

Consider another scenario when the solid surface is covered by vapors. The immersional wetting enthalpy, \( \Delta_W H \), is smaller than \( \Delta_{imm}H \) that describes the immersional enthalpy of a solid surface in equilibrium with vacuum. And the relationship between \( \Delta_{imm}H \) and \( \Delta_W H \) is given by the following equation,

Equation 5.8: \( \Delta_{imm}H = \Delta_W H + \Delta_{ads}H \)

where \( \Delta_{ads}H \) is the enthalpy of adsorption. \( \Delta_W H \) can be obtained, in the case of perfect wetting (i.e. \( \cos \theta = 1 \)), by the following equation,

Equation 5.9: \( \Delta_W H = \gamma_{SL} - \gamma_{SV} - T \left( \frac{\partial (\gamma_{SL} - \gamma_{SV})}{\partial T} \right)_p = \gamma_{LV} - T \left( \frac{\partial \gamma_{LV}}{\partial T} \right)_p = H_L \)

where \( H_L \) is the surface enthalpy of the liquid and for water \(^{336} \), with a value of 118 mJ/m\(^2\).
Different from adsorption and immersion, adhesion (i.e. adhesional wetting) occurs when a solid and a liquid interface are brought into contact. The change of Gibbs free energy, $\Delta_{adh}G$, is then,

**Equation 5.10:** $\Delta_{adh}G = \gamma_{SL} - (\gamma_S + \gamma_L)$

And the enthalpy of adhesion, $\Delta_{adh}H$, can be calculated by Equation 5.11

**Equation 5.11:** $\Delta_{adh}H = \Delta_{imm}H - H_L$

### 5.6.2 Water vapor adsorption experiments

The vapor adsorption isotherms of water on different nanoparticle powders are reported in Figure 5.4. Most adsorption isotherms except for C₆₀ and Ag-CIT were type II adsorption according to IUPAC classification, which suggested a relatively strong adsorbate-adsorbent interaction. The adsorption isotherms at low relative pressure indicated typical non-porous surfaces and formation of monolayer adsorption. At higher relative pressure, multilayer of water vapor began to form, and the condensation started between 0.6 – 0.8 for different nanoparticle powders. BET adsorption isotherm was applied to the low vapor pressure range ($0.05 < P/P_0 < 0.4$) to calculate the $C_{BET}$ solid-liquid interaction constant. These results are summarized in Table 5.5. Since $C_{BET}$ describes the affinity between the vapor and the solid surface, the smaller $C_{BET}$ indicates more hydrophobic surface. Thus from Table 5.5, it is clear that Ag-PVP and Ag-GA were more hydrophobic than fullerol, which was consistent with what other characterization methods suggested in this chapter. The manufacturer claimed that the
coating of SiO₂ made TiO₂ “super hydrophobic”, which seemed not the case judging from our results, as C_{BET} of TiO₂-SiO₂ and TiO₂ were similar.

Figure 5.4: Adsorption isotherms of water vapor adsorption.
(a) C₆₀ and Au-CIT; (b) Fullerol and Ag-CIT; (c) Ag-PVP and Ag-GA; and (d) TiO₂ and TiO₂-SiO₂
Table 5.5: Parameters obtained from water vapor adsorption and BET surface area measurement with N$_2$.

<table>
<thead>
<tr>
<th>NPs</th>
<th>C$_{60}$</th>
<th>Fullerol</th>
<th>Ag-CIT</th>
<th>Ag-PVP</th>
<th>Ag-GA</th>
<th>Au-CIT</th>
<th>TiO$_2$</th>
<th>TiO$_2$-SiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{BET}$</td>
<td>N/A</td>
<td>3.00</td>
<td>N/A</td>
<td>1.66</td>
<td>2.62$^a$</td>
<td>2.59</td>
<td>3.29</td>
<td>3.19</td>
</tr>
<tr>
<td>SSA$_w$(m$^2$/g)$^b$</td>
<td>N/A</td>
<td>51.7</td>
<td>N/A</td>
<td>13.6</td>
<td>22.0</td>
<td>0.5</td>
<td>30.3</td>
<td>4.5</td>
</tr>
<tr>
<td>SSA$_N$(m$^2$/g)$^c$</td>
<td>1.9</td>
<td>12.5</td>
<td>0.6</td>
<td>1.4</td>
<td>1.7</td>
<td>1.2</td>
<td>48.1</td>
<td>11.6</td>
</tr>
<tr>
<td>$\pi_{s}$(mJ/m$^2$)</td>
<td>5</td>
<td>110</td>
<td>330</td>
<td>85</td>
<td>231</td>
<td>214</td>
<td>102</td>
<td>105</td>
</tr>
</tbody>
</table>

$^a$ C$_{BET}$ was obtained by applying BET adsorption isotherm to 0.05 < P/P$_0$ < 0.3;  
$^b$ Specific surface area measured by water vapor adsorption;  
$^c$ Specific surface area measured by N$_2$ adsorption;

Meanwhile, the quantity of adsorbed water vapor in monolayer can also be calculated by fitting adsorption isotherm with BET equation. Assuming that the cross sectional area of adsorbed water vapor molecule is 0.106 nm$^2$, the specific surface area (SSA) of nanoparticle powders can be obtained (Table 5.5). Large discrepancies between SSA measured by water vapor and N$_2$ adsorption were found (Table 5.5), which might be attribute to the cross sectional area of water vapor molecule assumed. Since in a water vapor molecule the distance between the two H atoms is 1.514 Å (the H-O bond length is 0.958 Å with a bond angle of 104.45°), and the orientation of water vapor molecule on the solid surface is unknown, the cross sectional area might vary greatly. In addition, the affinity between water vapor molecules and the adsorption sites on the solid surface might be different with that between nitrogen gas molecule and solid surface. As a result, a monolayer of water vapor consists more molecules than a monolayer of nitrogen gas. Nonetheless, the SSA obtained from water vapor adsorption was used for our following discussion.
Based on the quantity of adsorbed water vapor in monolayer, the number of layers can be calculated. As the relative pressure increased, more layers of water vapor adsorbed onto the solid surface (Figure 5.5). Though the film structure for different nanoparticle powders was different at higher relative pressure, the formation of monolayer was statistically completed between 0.3 and 0.4 for most of them.

![Figure 5.5: Number of adsorbed layers of water vapor on different nanoparticle powders under different relative pressure.](image)

The water vapor adsorption isotherm on C_{60} powders, on the other hand, showed type III adsorption indicating the interaction between water vapor and C_{60} was weak. No monolayer of water vapor was formed at the low relative pressure, and the condensation started at P/P_0 = 0.8. From these result, it is safe to conclude that C_{60} is much more hydrophobic than those nanoparticles with type II water vapor adsorption. Considering the very hydrophobic nature of pristine C_{60}, it is not surprising that very
little water adsorbing onto its surface. The type II adsorption of water vapor on fullerene surface reported by Labille et al.\cite{Labille340} was not observed in this study, suggesting that the hydration of fullerene might not have occurred in our experiments. SSA of C$_{60}$ powders measured by N$_2$ adsorption was 1.9 m$^2$/g, similar with those reported elsewhere\cite{Labille340}.

As for Ag-CIT, the water vapor adsorption isotherm appeared to be type VI, showing a step formation of multilayers that usually occurs when the temperature is near the melting point of the adsorbate\cite{182}. However, the experimental results revealed that the adsorbed amount of water vapor experienced decreasing at the medium relative pressure, rather than a slow increasing that resembles a step formation of multilayers. The reason for the loss of water vapor from solid surface in the midst of an increasing relative pressure is unclear. It might be because the surface area was reduced due to dissolution of Ag or the desorption of citrate during the process. As a result, BET adsorption isotherm cannot be applied to Ag-CIT for calculating those parameters listed in Table 5.5.

Using the water vapor adsorption isotherms and Equation 5.3, the equilibrium film pressures ($\pi_e$) of water on the surface of different nanoparticle powders were obtained by integration (Table 5.5). $\pi_e$ will be used, together with results from immersion microcalorimetry, for the calculation of Gibbs free energies, enthalpies and entropies of the water-nanoparticle interaction.
5.6.3 Immersion microcalorimetry

In Table 5.6, the immersion enthalpy ($\Delta_{imm}H$) of different nanoparticle powders in water and octanol are reported. The value of enthalpy was normalized to the BET SSA measured by water vapor adsorption experiments. A negative enthalpy indicates that the immersion is exothermic, while positive enthalpy suggests endothermic immersion. Surprisingly, most of $\Delta_{imm}H$ for nanoparticles, except for C$_{60}$, were too large to be used to calculate the contact angle by Equation 5.7. The reason for this anomaly large $\Delta_{imm}H$ might be because that the measured enthalpy change accompanying the immersion process not only include the immersion enthalpy, but also the enthalpy of hydration, which led to irreversible change of surface structure. Another possible explanation for this phenomenon is that once mixed with solvent, the nanoparticle powders were dispersed into finer particles and the total surface area was drastically enlarged. This was supported by the fact that when fullerol powders were mixed with water, they quickly dissolved in the water and the size was around 100 nm. In this case, the premises for the derivation of Equation 5.7 are no longer valid. Thereby, Equation 5.7 cannot be used to calculate contact angle.

With $\Delta_{imm}H$ in water (Table 5.6), $\pi_e$ of water (Table 5.5) and surface enthalpy of water ($H_s = 118 \text{ mJ/m}^2$), the Gibbs free energies, enthalpies and entropies of adsorption, adhesion and immersion can be calculated based on Equation 5.5, 5.8, 5.10, 5.11 and $\gamma_{\text{water}} = 72 \text{ mJ/m}^2$. The results are summarized in Table 5.7.
Table 5.6: Immersion enthalpy (mJ/m$^2$) of different nanoparticle powders in water and octanol.

<table>
<thead>
<tr>
<th>NPs</th>
<th>C$_{60}$</th>
<th>Fullerol</th>
<th>Ag-CIT</th>
<th>Ag-PVP</th>
<th>Ag-GA</th>
<th>Au-CIT</th>
<th>TiO$_2$</th>
<th>TiO$_2$-SiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta_{imm} H$ (Water)</td>
<td>31</td>
<td>638</td>
<td>-2763</td>
<td>-391</td>
<td>-772</td>
<td>93</td>
<td>-518</td>
<td>-891</td>
</tr>
<tr>
<td>$\Delta_{imm} H$ (Octanol)</td>
<td>214</td>
<td>-15</td>
<td>16</td>
<td>238</td>
<td>-24</td>
<td>86</td>
<td>-138</td>
<td>-379</td>
</tr>
</tbody>
</table>

Table 5.7: Gibbs free energies, enthalpies and entropies of immersion, adsorption and adhesion between water and different nanoparticle powders (units of all the data are mJ/m$^2$) at $T = 298$ K.

<table>
<thead>
<tr>
<th></th>
<th>C$_{60}$</th>
<th>Fullerol</th>
<th>Ag-CIT</th>
<th>Ag-PVP</th>
<th>Ag-GA</th>
<th>Au-CIT</th>
<th>TiO$_2$</th>
<th>TiO$_2$-SiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta_{imm} H$</td>
<td>31</td>
<td>638</td>
<td>-2763</td>
<td>-391</td>
<td>-772</td>
<td>93</td>
<td>-518</td>
<td>-891</td>
</tr>
<tr>
<td>$\Delta_{imm} G$</td>
<td>-77</td>
<td>-182</td>
<td>-402</td>
<td>-157</td>
<td>-303</td>
<td>-286</td>
<td>-174</td>
<td>-177</td>
</tr>
<tr>
<td>$T\Delta_{imm} S$</td>
<td>108</td>
<td>820</td>
<td>-2361</td>
<td>-234</td>
<td>-469</td>
<td>379</td>
<td>-344</td>
<td>-714</td>
</tr>
<tr>
<td>$\Delta_{ads} H$</td>
<td>149</td>
<td>756</td>
<td>-2645</td>
<td>-273</td>
<td>-654</td>
<td>211</td>
<td>-400</td>
<td>-773</td>
</tr>
<tr>
<td>$\Delta_{ads} G$</td>
<td>-5</td>
<td>-110</td>
<td>-330</td>
<td>-85</td>
<td>-231</td>
<td>-214</td>
<td>-102</td>
<td>-105</td>
</tr>
<tr>
<td>$T\Delta_{ads} S$</td>
<td>154</td>
<td>866</td>
<td>-2315</td>
<td>-188</td>
<td>-423</td>
<td>425</td>
<td>-298</td>
<td>-668</td>
</tr>
<tr>
<td>$\Delta_{adh} H$</td>
<td>-87</td>
<td>520</td>
<td>-2881</td>
<td>-509</td>
<td>-890</td>
<td>-25</td>
<td>-636</td>
<td>-1009</td>
</tr>
<tr>
<td>$\Delta_{adh} G$</td>
<td>-149</td>
<td>-254</td>
<td>-474</td>
<td>-229</td>
<td>-375</td>
<td>-358</td>
<td>-246</td>
<td>-249</td>
</tr>
<tr>
<td>$T\Delta_{adh} S$</td>
<td>62</td>
<td>774</td>
<td>-2407</td>
<td>-280</td>
<td>-515</td>
<td>333</td>
<td>-390</td>
<td>-760</td>
</tr>
</tbody>
</table>

C$_{60}$ had the smallest $\Delta_{imm} G$, $\Delta_{ads} G$, and $\Delta_{adh} G$ of all the nanoparticle powders tested, which suggested that the interaction between water and C$_{60}$ was weaker and thus C$_{60}$ was more hydrophobic. Even though the results for C$_{60}$ were consistent with other characterization methods, it was not the case for other nanoparticles. For example, fullerol, as the most hydrophilic one by previous results, failed to develop the largest interactions with water as shown in Table 5.6. TiO$_2$-SiO$_2$, which was expected to be more hydrophobic than TiO$_2$, had a stronger interaction with water than TiO$_2$. The seemingly contradictory results in Table 5.6 might be resulting from the change of surface area.
during the immersion process, as the aggregation states of nanoparticles changed. An
evidence for this is that the SSA of nanoparticles in aqueous solution, calculated based
on the particle size obtained from TEM, was different with what was measured by BET
method. In addition, Ag-PVP and Ag-GA powders were prepared by freeze-drying
corresponding solution. There might be a number of dried polymers in the nanoparticle
powders, and the desorption or hydration of these polymers in water might contribute
to the overall immersion enthalpy. At the relatively short time scale of the immersion
experiment (5 – 10 min), transformations of nanoparticle such as dissolution were not
expected to occur. Therefore, they were not likely a factor in the abnormal results.
However, during the freeze-drying process and the storage of freeze-dried samples, the
surface of nanoparticle surface might undergo some kinds of chemical reaction such as
oxidation, even though the freeze-drying process was conducted under vacuum. To sum
up, from the experimental results shown in Table 5.6, the immersion microcalorimetry
and the calculation of $\Delta_{imm} G$, $\Delta_{ads} G$, and $\Delta_{adh} G$ might not be a good indicator of the
hydrophobicity for nanoparticles. Other than the possible interferences discussed above,
even a hydrophobic C$_{60}$ was shown to be thermodynamically favorable to interact with
water by this method (Table 5.6).

### 5.6.4 Thermogravimetric analysis of water desorption

The TGA result of C$_{60}$, fullerol, Ag-PVP and Ag-GA are shown in Figure B1-B4,
and results of other nanoparticle powders are reported in Appendix A. It is believed that
the loss of water up to 120°C under nitrogen in the TGA analysis was physically adsorbed and depends on the humidity in sample preparation, while the loss of weight up to 500°C was chemically bond water and functional groups that had strong affinity for water, such as hydroxyl and carboxyl groups on the solid surface. From Figure B1, under nitrogen, there was minimal weight loss for C₆₀, most of which was physically adsorbed water; while under nitrogen, majority of the mass of C₆₀ was burnt out at high temperature (>500°C). As for fullerol, much more chemically adsorbed water was removed between 120°C and 500°C than physically adsorbed water, and the hydroxyl groups on its surface was responsible for this. For Ag-PVP and Ag-GA, similar weight losses of both physically and chemically adsorbed water were observed; while the pyrolysis of residual polymers might also contribute to the decreasing weight. However, after the ambient gas was switched from nitrogen to oxygen, the weight of both silver nanoparticle with polymeric coatings experienced a sudden drop and then increased. The burning of residual polymers might be the reason for the large weight loss, and the gain of weight might be due to the oxidation of silver. The slow oxidation of silver after the polymers were burnt out suggested that the presence of polymers on the nanoparticle surface shield them from oxidation not only in water, but also during the freeze-drying process.
Based on an equation proposed by Anderson and Klinowski \(^{191}\), which utilized the loss of weight at different temperature during the TGA analysis, Equation 5.12 was used to calculate an index, H, to indicate the hydrophobicity of nanoparticle powders.

**Equation 5.12:**

\[
H = \frac{\text{Weight loss up to } 120^\circ\text{C}}{\text{Weight loss up to } 500^\circ\text{C}}
\]

It should be pointed out that the weight loss to apply to Equation 5.12 was measured under nitrogen as discussed previously. Thus, a larger H indicates a more hydrophobic surface compared to lower H. The results are summarized in Table 5.8. However, based on our results, H seemed not to be a good indicator of hydrophobicity as shown in Table 5.8. For example, H of more hydrophobic TiO\(_2\)-SiO\(_2\) was smaller than that of TiO\(_2\). The more hydrophilic Ag-CIT had a similar H with Ag-PVP and Ag-GA. Judging from our results in Table 5.8, the percentage of weight loss in the range of 120°C-500°C appeared to be a better indicator of hydrophobicity, though as discussed earlier, the pyrolysis of residual polymers might also be part of the overall weight loss in this temperature range. While being one of the least hydrophobic nanoparticles according to previous results, Au-CIT had a rather small weight loss in the range of 120°C-500°C, possibly because Au-CIT powders were commercial products that might have a different initial humidity compared with those prepared by freeze-drying.

Assuming the weight loss in the range of 120°C-500°C was associated with hydroxyl group (OH), the OH density on the surface of nanoparticle powders can be calculated by Equation 5.13 \(^{190}\).
Table 5.8: Hydrophobicity index, H, as calculated from Equation 5.12 and percentage of weight loss of nanoparticles by TGA.

<table>
<thead>
<tr>
<th>NPs</th>
<th>C₆₀</th>
<th>Fullerol</th>
<th>Ag-CIT</th>
<th>Ag-PVP</th>
<th>Ag-GA</th>
<th>Au-CIT</th>
<th>TiO₂</th>
<th>TiO₂-SiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss up to 120°C (%)</td>
<td>0.3</td>
<td>2.4</td>
<td>5.4</td>
<td>0.3</td>
<td>1.6</td>
<td>0.6</td>
<td>1.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Weight loss 120°C-500°C (%)</td>
<td>0.1</td>
<td>21.2</td>
<td>20.0</td>
<td>2.6</td>
<td>8.8</td>
<td>0.3</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>OH density (nm⁻²)</td>
<td>22</td>
<td>280</td>
<td>696</td>
<td>127</td>
<td>271</td>
<td>533</td>
<td>41</td>
<td>133</td>
</tr>
</tbody>
</table>

Equation 5.13: \[\#OH = \frac{M_{120}-M_{500} \times N_A \times 2}{MW \times SSA \times M_{120}}\]

where \(M_{120}\) and \(M_{500}\) were the weight of sample at 120°C and 500°C, respectively, \(MW\) was the molecular weight of water, \(N_A\) was the Avogadro’s constant, and SSA was the specific surface area of sample. These results are also reported in Table 5.8. Since the OH density on the surface of a single molecule C₆₀(OH)₂₄ is approximately 8, the rather large OH density in Table 5.8 suggested that other mechanisms, other than the removal of hydroxyl groups, were responsible for the weight loss in the range of 120°C-500°C.

5.7 Heterogeneity in surface hydrophobicity as revealed by selective fluorescence labeling

After the hydrophilic region on the surface was selectively labeled with FITC, the images of fullerene, fullerol, Ag-PVP and TiO₂, observed by fluorescence microscope and optical microscope, were shown in Figure 5.6. The distribution of hydrophobic and hydrophilic region on their surfaces is completely different. For fullerene, whose surface is very hydrophobic, almost nothing was observed from the fluorescence micrograph.
For fullerol and TiO$_2$ that are hydrophilic, there was no appreciable difference between fluorescence and optical images in shape. For Ag-PVP, some particles were not fluorescent at all, like fullerene, while some were partially or completely fluorescent.

Figure 5.6: Fluorescence and optical micrographs of nanoparticles. (a) Fullerene; (b) Fullerol; (c) Ag-PVP; and (d) TiO$_2$. 
Confocal fluorescent microscopy results of Ag-PVP were shown in Figure 5.7, which confirmed the patchy fluorescent region on the Ag-PVP surface. This result demonstrated the heterogeneity in surface hydrophobicity, and suggested that the coatings on the particle surface were not uniformly distributed.

![Confocal fluorescent micrographs of APTS-FITC-modified Ag-PVP.](image)

**Figure 5.7**: Confocal fluorescent micrographs of APTS-FITC-modified Ag-PVP.

The fluorescent ratios of seven different nanoparticles were summarized in Table 5.9. For each nanoparticle, at least 100 images of particle were collected and analyzed. The surfaces of fullerene, fullerol and CeO$_2$ were the most homogeneous in terms of hydrophobicity, with fullerene being almost completely hydrophobic while fullerol and CeO$_2$ hydrophilic. The surfaces of other nanoparticles, such as Ag-PVP, Ag-GA, TiO$_2$ and ZnO, were heterogeneous to a certain degree as their fluorescent ratio fell in
between 0 and 1. The relative hydrophobicity of fullerene, fullerol, Ag-PVP and Ag-GA was consistent with the results from other quantitative characterization methods tested previously.

Table 5.9: Fluorescent ratios of different nanoparticles by selective fluorescence labeling.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Fluorescent ratio</th>
<th>Number of particles counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fullerene</td>
<td>0.03</td>
<td>143</td>
</tr>
<tr>
<td>Fullerol</td>
<td>0.97</td>
<td>187</td>
</tr>
<tr>
<td>Ag-PVP</td>
<td>0.26</td>
<td>210</td>
</tr>
<tr>
<td>Ag-GA</td>
<td>0.38</td>
<td>182</td>
</tr>
<tr>
<td>TiO2</td>
<td>0.66</td>
<td>306</td>
</tr>
<tr>
<td>ZnO</td>
<td>0.93</td>
<td>378</td>
</tr>
<tr>
<td>CeO2</td>
<td>0.95</td>
<td>462</td>
</tr>
</tbody>
</table>

5.8 Summary of results

Figure 5.8 shows the comparison of the experimental results, which can be used as indicators for hydrophobicity, from different characterization methods discussed previously. The order of hydrophobicity for six tested nanoparticles are consistent across different testing procedures, except for some minor discrepancies as explained in previous sections. It confirms that the methods explored in this work provided a largely coherent description of the relative surface hydrophobicity for the NPs tested.

Figure 5.9 shows the pair-wise comparison of these indicators and Table 5.10 summarizes the pairwise correlation coefficients. From Figure 5.9, it is observed that the organic dye adsorption method provides a consistent evaluation of hydrophobicity with other methods. The naphthalene adsorption method appears to not as good as organic
dye adsorption, and it is because of the abnormal adsorption of naphthalene to fullerol aforementioned. Except for fullerol, using naphthalene as a molecular probe yields consistent assessment of hydrophobicity with other methods (data not shown).

![Figure 5.8: Comparison of experimental results from different characterization methods for the surface hydrophobicity of nanoparticles](image)

**Table 5.10: Pairwise correlation coefficients for different indicators of hydrophobicity.**

<table>
<thead>
<tr>
<th></th>
<th>AdsRB</th>
<th>AdsNPTL</th>
<th>K_{ow}</th>
<th>CA</th>
<th>cBET</th>
<th>TGA</th>
<th>immH</th>
<th>adsG</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdsRB</td>
<td>1</td>
<td>0.1691</td>
<td>0.905</td>
<td>0.85</td>
<td>-0.901</td>
<td>-0.8393</td>
<td>0.149</td>
<td>0.537</td>
</tr>
<tr>
<td>AdsNPTL</td>
<td>0.169</td>
<td>1</td>
<td>0.494</td>
<td>0.427</td>
<td>-0.394</td>
<td>0.0408</td>
<td>0.616</td>
<td>0.77</td>
</tr>
<tr>
<td>K_{ow}</td>
<td>0.905</td>
<td>0.4945</td>
<td>1</td>
<td>0.978</td>
<td>-0.838</td>
<td>-0.6698</td>
<td>0.231</td>
<td>0.642</td>
</tr>
<tr>
<td>CA</td>
<td>0.85</td>
<td>0.4274</td>
<td>0.978</td>
<td>1</td>
<td>-0.722</td>
<td>-0.6188</td>
<td>0.107</td>
<td>0.505</td>
</tr>
<tr>
<td>cBET</td>
<td>-0.901</td>
<td>-0.3943</td>
<td>-0.838</td>
<td>-0.722</td>
<td>1</td>
<td>0.6804</td>
<td>-0.292</td>
<td>-0.777</td>
</tr>
<tr>
<td>TGA</td>
<td>-0.839</td>
<td>0.0408</td>
<td>-0.67</td>
<td>-0.619</td>
<td>0.68</td>
<td>1</td>
<td>-0.374</td>
<td>-0.466</td>
</tr>
<tr>
<td>immH</td>
<td>0.149</td>
<td>0.6162</td>
<td>0.231</td>
<td>0.107</td>
<td>-0.292</td>
<td>-0.3743</td>
<td>1</td>
<td>0.74</td>
</tr>
<tr>
<td>adsG</td>
<td>0.537</td>
<td>0.77</td>
<td>0.642</td>
<td>0.505</td>
<td>-0.777</td>
<td>-0.4655</td>
<td>0.74</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 5.9: Pairwise comparison of experimental results from different characterization methods for the surface hydrophobicity of nanoparticles.

(a) AdsRB: the slope of the adsorption isotherm for hydrophobic dye RB and the negative value of the slope of adsorption isotherm for hydrophilic dye NB; (b) AdsNPTL: Freundlich constant calculated from the adsorption isotherm of naphthalene; (c) Kow: octanol-water partition coefficient; (d) CA: contact angle measured on thin films of nanoparticles; (e) cBET: BET constant calculated from the water vapor adsorption isotherm; (f) TGA: the weight loss (%) of nanoparticles measured by TGA in the range of 120°C-500°C; (g) immH: the immersion enthalpy in water; and (h) adsG: Gibbs free energy of water vapor adsorption on nanomaterial powders. For indicators (a), (b), (c), (d), (g) and (h), the larger the indicator, the more hydrophobic the nanoparticle is; and for indicators (e) and (f), the smaller the indicator, the more hydrophobic the nanoparticle is.
Indeed, concerns as to whether or not $K_{ow}$ is a suitable notion for NPs $^{12}$ reflect a violation in key assumptions that tested substances diffuse freely between the phases, and this assumption is likely compromised in the case of NPs. Our results show that despite these limitations, the trends in $K_{ow}$ are largely consistent with other measures of NP hydrophobicity.

The pairwise comparison shown in Figure 5.9 and Table 5.10 suggests that the immersion enthalpy and Gibbs free energy of water vapor adsorption are not as good indicators for hydrophobicity as adsorption of molecular probes and $K_{ow}$. As discussed in previous sections, characterization methods that applied to nanomaterial powders introduce physical or chemical transformations to the nanoparticle surface, which limits a rigorous thermodynamic interpretation of the results. Thus, thermodynamic indicators like immersion enthalpy and adsorptive free energy are not ideal in this case.

It should be pointed out that the intent of this research was not to quantitatively measure the surface hydrophobicity of nanoparticles, but rather to qualitatively characterize hydrophobicity and rank nanoparticles by their relative hydrophobicity. Overall, each of the methods evaluated in this study has their advantages and shortcomings. However, based on the consistency with other methods and the more solid grounds for interpretation of the results, it is concluded that the in-situ adsorptive method by molecular probes offers some advantages over the other methods.
6 Effect of surface hydrophobicity on the attachment of nanoparticles to bacterial surface

6.1 Introduction

The attachment of nanoparticles to the bacterial surface is a crucial part of an adsorption, distribution, metabolism and excretion (ADME) analysis that is often conducted in the ecotoxicity studies on hazardous materials. It serves as the first step for potential uptake of nanoparticles by the cells. The presence of nanoparticles on the exterior surface of bacteria might impair cellular functions, as well as causing the cell membrane damage.

Since the bacterial surface is the outermost boundary separating the cell from the ambient environment, the surface structure and chemistry is important in determining the interaction between nanoparticles and bacteria. The surfaces of Gram-positive and Gram-negative bacteria represent two different types of surface structure for bacteria. The cell wall of Gram-positive consists of a thick peptidoglycan layer; while for Gram-negative bacteria, a thinner peptidoglycan layer is covered by an additional peptidoglycan layer with LPS anchored. Most bacteria are usually surrounded by EPS, a complex matrix of bio-polymers. The components of EPS, which include proteins, polysaccharides and humic substances, often control the physicochemical properties of bacterial surface. Depending on their life form, bacteria can also be divided in two classes, planktonic and attached (i.e. biofilm). Biofilm is an uninterrupted multilayer of
bacterial cells that accumulate at a living or inert surface and are surrounded by a self-developed matrix of EPS \(^{227}\).

Biofilm is often overlooked in studies that focus on the transport of nanoparticles in well-defined porous media used to represent those ENPs might encounter in the natural aquatic system such as the soil matrix. Biofilm is ubiquitous in natural and engineered environments and are commonly found in soils and at water-sediment interfaces as coatings surround particles \(^{21}\). Surprisingly, little attention has been paid to studying the impact biofilm makes to the fate and transport of ENPs in a biofilm-laden porous media \(^{22-26}\). In these limited number of studies, *Pseudomonas aeruginosa* (PA) biofilm was found to reduce the mobility of functionalized polystyrene latex nanoparticles by Tripathi *et al* \(^{22}\), nanosized laponite clay particles by Leon-Morales *et al* \(^{24}\), and NZVI particles by Lerner *et al* \(^{25}\). The transport of fullerene \(C_{60}\) nanoparticles was retarded by biofilms formed by *Escherichia coli* (E. coli) \(^{26}\). The surface potential alone, thus the electrical double layer (EDL) interaction, could not explain the altered mobility \(^{22,26}\). The hydrophobic interaction \(^{26}\) and steric interaction (repulsion and bridging) \(^{25}\) were proposed as candidates that affect the attachment of nanoparticles to the collector surface. Nevertheless, because of the complex nature of the biofilm as well as the biofilm-coated surface, the governing mechanism for the transport of nanoparticles in biofilm-grown porous media is still largely unknown.
EPS, responsible for 85% of the biofilm mass\textsuperscript{227}, mediate attachment of bacteria to surfaces, and aid in the formation and integrity of biofilm structure\textsuperscript{343}. The composition of biofilm (i.e. type of macromolecules, concentration of macromolecules) can be completely different for different species of bacteria and growth condition\textsuperscript{227}. For instance, the primary polysaccharides constituting the EPS matrix produced by Gram-positive and Gram-negative bacteria were known to be different\textsuperscript{81}. And the amount of polysaccharides and the ratio of cells/EPS could vary depending on whether the biofilm was grown in a sand column or on agar plates\textsuperscript{24}. The composition of EPS, especially the ratio of proteins/polysaccharides, was shown to correlate with the deposition of nanoparticles on biofilm-coated surface\textsuperscript{24,228}. Morrow et al\textsuperscript{344} revealed that quantum dots preferentially colocalized with extracellular protein rather than other components or cell surface in a PA biofilm by confocal laser scanning microscopy. Though the affinity of nanoparticles to EPS was not necessarily a good predictor for biofilm\textsuperscript{22}, studying the interaction between nanoparticles and EPS components could still yield useful information on how biofilm affect the transport of nanoparticles.

Though limited, several studies have been done to investigate the attachment of nanoparticles to planktonic bacterial surface\textsuperscript{279,282}. Physicochemical properties such as size, shape, surface hydrophobicity and coatings are factors that affecting the nanoparticle-bacteria interaction\textsuperscript{283-285}. The surface chemistry of bacteria is also suggested as influential to the interaction with nanoparticles, for example, the
attachment of nanoparticles to Gram-positive and Gram-negative bacteria were found to be different \(^{287-288}\).

In this study, the mobility of selected ENPs in the granular porous media in the presence of biofilms was investigated by column experiments using glass beads coated with gram-negative and gram-positive bacteria. In addition, bovine serum albumin (BSA) and alginate coatings were employed as surrogate for proteins and polysaccharides to evaluate their impact on the transport behavior of ENPs. Selected ENPs include carbon-based and silver-based nanoparticles with or without polymeric coatings, and they were different in surface hydrophobicity so that the effect of hydrophobic and steric interactions can be studied. The influence of divalent ions (i.e. \(\text{Ca}^{2+}\)) to the surface property of biofilm and to the retention of ENPs was also investigated so as to understand the impact of environmental conditions to the transport behavior of ENPs in biofilm-laden porous media. The attachment of nanoparticles to planktonic bacterial surface with and without EPS was also studied, and the attachment at different concentration of nanoparticles was fitted to Langmuir adsorption isotherm. The effect of surface hydrophobicity, as well as pH and ionic strength was evaluated. Thin-section TEM was used to reveal the attachment of nanoparticles to the planktonic bacterial surface.
6.2 Characteristics of nanoparticle suspensions

Six different engineered nanoparticle aqueous suspensions were studied in this work: aqu-nC$_{60}$, fullerol, Ag-CIT, Ag-PVP, Ag-GA, and Au-CIT. In Table 6.1, the main characteristics of nanoparticles relevant to this study were summarized. Surface hydrophobicity was characterized by organic dye adsorption method that is also briefly described in section 3.5.3.

Table 6.1: Characteristics of nanoparticle suspensions.

<table>
<thead>
<tr>
<th>NPs</th>
<th>Size$^a$/nm</th>
<th>Size$^b$/nm</th>
<th>EPM/$^{10^{-8}}$m$^2$/V.s</th>
<th>$\zeta$-potential/mV</th>
<th>Hydrophobicity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqu-nC$_{60}$</td>
<td>102.3±28.9</td>
<td>88±41</td>
<td>-2.480±0.024</td>
<td>-31.66±0.31</td>
<td>RB, 0.24$^e$</td>
<td>Lab prepared</td>
</tr>
<tr>
<td>Fullerol</td>
<td>105.1±29.4</td>
<td>97±39</td>
<td>-4.527±0.031</td>
<td>-57.79±0.39</td>
<td>NB, 0.16</td>
<td>Lab prepared</td>
</tr>
<tr>
<td>Ag-CIT</td>
<td>26.5±12.7</td>
<td>19±4</td>
<td>-3.329±0.121</td>
<td>-42.49±1.54</td>
<td>NB, 0.08</td>
<td>CEINT$^g$</td>
</tr>
<tr>
<td>Ag-PVP</td>
<td>39.6±10.8</td>
<td>36±9</td>
<td>-0.765±0.020</td>
<td>-9.77±0.25</td>
<td>RB, 0.08</td>
<td>CEINT</td>
</tr>
<tr>
<td>Ag-GA</td>
<td>38.4±14.6</td>
<td>32±6</td>
<td>-0.425±0.028</td>
<td>-5.43±0.36</td>
<td>RB, 0.06</td>
<td>CEINT</td>
</tr>
<tr>
<td>Au-CIT</td>
<td>27.4±7.8</td>
<td>14±5</td>
<td>-2.578±0.042</td>
<td>-32.92±0.54</td>
<td>RB, 0.02</td>
<td>CEINT</td>
</tr>
</tbody>
</table>

$^a$ The second order average hydrodynamic diameter given by DLS ± standard deviation;
$^b$ Size determined by TEM image analysis, all values are means ± SD;
$^c$ All values are means ± 95% confidence interval (n = 3);
$^d$ Surface hydrophobicity measured by Rose Bengal (RB) adsorption (SI);
$^e$ The slope of PQ versus total surface area;
$^f$ Surface hydrophobicity measured by Nile Blue (NB) adsorption (SI);
$^g$ NPs suspension was obtained from sample store of Center for the Environmental Implications of Nano-Technology (CEINT, Durham, NC, USA)

6.3 Characterization of porous media

The spatial distribution of biofilm on GB in the column was shown in Figure 6.1. The total biomass per unit mass of GB in different section of the column was not evenly distributed for either PA or BC; however, the similar pattern followed for both bacteria.
in that less biofilm grew in the middle of the column compared to either end. The relatively limited availability to oxygen and nutrient deep inside the column likely contributed to this uneven growth of biofilm. BC was a facultative anaerobe, while PA was classified as an aerobic organism. Therefore, the difference in biomass between the middle and end of column for BC was less pronounced than PA. Nonetheless, the flow direction switching while the formation of biofilm led to a relatively homogeneous porous media in terms of the biofilm distribution.

Figure 6.1: Spatial distribution of biofilm in the column.
Error bars represent ±1 standard deviation.

The coverage of biofilm on the surface of GB was shown by SEM in Figure 6.2. Though a large portion of the GB surface was coated by biofilm, it was evident that the distribution of biofilm was not homogeneous. The bacteria were imbedded in a matrix of polymer-like substances.
Figure 6.2: SEM images taken from clean and biofilm-coated GB at different magnifications.

Clean GB at (a) 500×, (b) 15000×; and biofilm-coated GB at (c) 500×, (d) 15000× and (e) 35000×
The EPM and ζ-potential measurement for different porous media was summarized in Table C2. The ζ-potential of all the porous media investigated was negative, with clean GB being the most negative surface (-66.6±3.8 mV). BC biofilm (-16.7±2.0 mV) was less negative than PA biofilm (-38.1±2.9 mV). This was as expected since the biofilms of gram-positive bacteria produced an EPS that was primarily cationic; thus part of the negative charges on bacteria surface was neutralized.

The quantitative analysis of different components of EPS in the biofilm showed (Figure C1) that there were more proteins (PT) than polysaccharides (PS) in the EPS of PA biofilm (PT/PS = 1.8); while in BC biofilm, EPS contained more polysaccharides (PT/PS = 0.5). Admittedly, the composition of EPS would vary as a result of the aging of the biofilm or the changing of environmental condition, but PT/PS ratio was clearly different between PA and BC at this phase of the biofilm growth.

BSA-coated GB was found to be the most hydrophobic collector surface of all those examined in this study, followed by PA biofilm, BC biofilm, and alginate-coated GB (Figure C2). No adsorption of RB onto clean GB was observed, suggesting the GB surface was hydrophilic. The presence of both hydrophilic (e.g. hydroxyl, carboxyl and phenolic) and hydrophobic functional groups (e.g. aromatics, aliphatics in proteins, and hydrophobic regions in carbohydrates) in EPS molecules suggests that the surface of biofilm is amphiphilic, and the relative hydrophobicity of biofilm is controlled by the composition of the EPS. The hydrophobic fraction of EPS was found to mainly
comprise proteins; while polysaccharides were the dominant macromolecules in the hydrophilic fraction of EPS\textsuperscript{237}. Our results were consistent with these previous reports.

6.4 \textit{Transport and retention of nanoparticles in biofilm coated porous media}

With the measured BTC and Equation 3.4, the attachment efficiency of nanoparticle to different collector surface was calculated (Figure 6.3). Apart from Ag-PVP, more nanoparticles were retained by biofilm than clean GB as shown by the relative larger $\alpha$, which suggested that the presence of biofilm in natural environment would affect the fate and transport of nanoparticles one way or the other. Considering the fact that biofilm consists of a complex matrix of cells and biopolymers and the adherence of biofilm on GB was spatially heterogeneous, attempts were not made to calculate the particle-collector surface interaction energy and to predict $\alpha$ using classic Derjaguin-Landau-Verwey-Overbeek (DLVO) theory based on some global parameters such as the $\zeta$-potential. Actually, Tripathi \textit{et al}\textsuperscript{22} and Lerner \textit{et al}\textsuperscript{25} have already shown that DLVO model with generalized parameters provided poor prediction to the experiment-measured $\alpha$. 
For aqu-nC₆₀, the retention on clean GB was the weakest of all the porous media, possibly because clean GB had the most negative charged surface thus the enhanced EDL interaction (Table C2). However, the difference in surface potential could not explain the difference in $\alpha$ for biofilm-coated GB, as BC biofilm with less negative surface yielded lower $\alpha$. This seemingly deviation from DLVO theory was also observed for BSA- and alginate-coated GB. These results demonstrated that, in addition to DLVO force, other interactions must be account for the particle-surface interaction and playing a major role in determining $\alpha$. With aqu-nC₆₀ being a moderately hydrophobic nanoparticle (Table 6.1), the hydrophobic interaction between aqu-nC₆₀ and hydrophobic region of biofilm would inevitably contribute to the overall particle-surface interaction. This was confirmed by the experimental results (Figure 6.3) that the most hydrophobic BSA-coated GB achieved the highest retention, while the least hydrophobic alginate-
coated GB had the lowest $\alpha$. Since the components of PA and BC biofilms were of
different combination of proteins and polysaccharides (Figure C1), the hydrophobicity,
as well as $\alpha$, of PA and BC biofilm fell in between BSA- and alginate-coated GB. Even
though at the ionic strength of 1 mM, the Debye length $\kappa$-1 was 9.61 nm so that the
hydrophobic functional groups in protein might not be long enough to protrude
through the EDL; the hydrophobic interaction was still effective at long range $^{32}$ thus
making the collector attractive to particles. Therefore, the hydrophobicity of biofilm, or
in other words the hydrophobic and hydrophilic region of biofilm, played a significant
role in deciding the mobility of hydrophobic nanoparticles in porous media with biofilm
grown inside. As shown in this study, proteins served as the source of hydrophobicity
for biofilm; thus the relative abundance of protein compared to the polysaccharides
components in the biofilm could be a useful predictor to the fate and transport of
hydrophobic nanoparticles. However, it should be pointed out that due to the complex
nature of biofilm which resulted from different species of bacteria (e.g. gram-positive or
gram negative), age of biofilm or environmental condition, the PT/PS ratio or even the
hydrophobicity of extracellular proteins might vary $^{228,237,348}$.

Although PA and BC biofilms, BSA and alginate on GB all attenuated the
mobility of fullerol in porous media to a certain degree, there was no statistical
difference in $\alpha$ among them. The correlation between hydrophobicity of collector surface
and attachment efficiency as indicated for the case of aqu-nC$_{60}$ was not observed.
Considering the hydrophilic property of fullerol (Table 6.1), the lack of hydrophobic interaction between nanoparticles and collectors was not surprising. The enhanced affinity might be attributed to the hydrogen bonding the hydroxyl groups on the surface of fullerol formed with the collector surface, or the capability of large polysaccharides to “collect” small nanoparticles.

Despite being of different core material, the transport of Ag-CIT in porous media consisting of clean GB or biofilm-grown GB followed similar pattern as fullerol in that $\alpha$ for clean GB was smaller than any other porous media tested. Like fullerol, the increased retention of Ag-CIT by biofilm or macromolecules was not a result of hydrophobic interaction as Ag-CIT was not deemed hydrophobic (Table 6.1). Similar mechanisms holding fullerol at the surface of biofilm might also involve in the silver nanoparticle-surface interaction, with residual citrate molecule on Ag-CIT providing sites for hydrogen bonding or other associations with macromolecules in biofilm.

In contrast to Ag-CIT, the presence of biofilm or alginate did not reduce the mobility of Ag-PVP, though these collectors were less negative than clean GB. Given that Ag-PVP nanoparticles were wrapped by a layer of PVP polymers and there were abundant biopolymers in biofilm, the steric repulsion between PVP and biopolymers was the most likely candidate to stabilize Ag-PVP. Numerous studies have revealed the prominent role steric interaction played in influencing the interaction between two polymer-covered surfaces. Lerner et al reported that under certain ionic strength,
the steric interaction could turn into attractive in the form of bridging between polymers

25; nevertheless in this study, the ionic strength was not large enough for this
phenomenon to occur. In spite of the steric repulsion, $\alpha$ for BSA-coated GB was
noticeably larger than that for clean GB, which might be attributed to the hydrophobic
interaction. This attractive interaction between slightly hydrophobic Ag-PVP (Table 6.1)
and proteins attenuated the effect of repulsive steric interaction and made the
attachment of Ag-PVP to collector surface a synergic result of both interactions. As a
matter of fact, $\alpha$ for alginate-coated GB was the smallest of all porous media evaluated.
And $\alpha$ for PA or BC biofilm was between that for BSA and alginate, in a similar way
with aqu-nC$_{60}$.

6.5 Effect of divalent cations on the transport of nanoparticles
in biofilm-coated porous media

After the pre-treatment by Ca$^{2+}$, the hydrophobicity of every GB with coatings
was increased, even though not to the same degree (Figure C3). The alginate-coated GB
and PA biofilm became significantly more hydrophobic after pre-treated by Ca$^{2+}$, and
the hydrophobicity of BSA-coated GB was also moderately enhanced. Yet, the increase
of hydrophobicity for BC biofilm was the least. The capability of acting as bridging
agents between negatively charge molecules or surfaces for divalent ions like Ca$^{2+}$ might
be the reason for this “hydrophobizing effect” to polysaccharides. By bridging
polysaccharide chains via Coulomb forces and altering the molecular conformation, a
greater number of internal hydrogen bonds were formed so that the overall
The hydrophilicity of polysaccharides was lessened. Gram-negative bacteria had more anionic polysaccharides in their biofilm matrix than gram-positive bacteria, thus the effect of Ca²⁺ on hydrophobicity was more pronounced for PA biofilm. As for proteins, it was demonstrated that Ca²⁺ specifically attached to the hydrophobic region of proteins and made them more hydrophobic. Moreover, Ca²⁺ was shown to have a hydrophobizing effect on surfaces whose hydrophilicity was related to the negatively charged surface. By any mechanism, all of the porous media with coatings were hydrophobized by Ca²⁺ as illustrated in Figure C3. In the nutrient solution used for biofilm growth, the concentration of monovalent ions (i.e. K⁺ and Na⁺) was approximately 50 mM, indicating that the monovalent ions could not achieve the similar hydrophobizing effect as divalent ions did.

The attachment efficiencies of nanoparticles to the Ca²⁺ treated porous media were summarized in Figure 6.4. The transport of all the nanoparticles was retarded compared to that in non-treated porous media. For aqu-nC₆₀, the difference in α was reduced in a similar pattern like the hydrophobicity of the four different biofilm- or macromolecules-coated GB. The most hydrophobic BSA-coated GB and least hydrophobic alginate-coated GB yielded the largest and smallest α, respectively, which suggested that the hydrophobic interaction still controlled the deposition of aqu-nC₆₀. In contrast to aqu-nC₆₀, the change of the mobility of hydrophobic Ag-PVP was not as dramatic. Although the charge neutralization, increasing hydrophobicity and the
bridging effect brought in by Ca\textsuperscript{2+} could potentially reduce the influence of steric interaction\textsuperscript{349}, it was still the major barrier for a stable attachment between two polymer-coated surfaces. Pre-treating porous media with coatings greatly elevated \( \alpha \) for both fullerol and Ag-CIT, either by the aforementioned charge neutralization effect of Ca\textsuperscript{2+}, or due to the gel formation initiated by the addition of Ca\textsuperscript{2+}\textsuperscript{2}.

Figure 6.4: Attachment efficiencies of selected nanoparticles on clean GB, PA and BC biofilm, BSA and alginate-coated GB after pre-treated by Ca\textsuperscript{2+}. Error bars represent \( \pm 1 \) standard deviation.

6.6 Attachment of nanoparticle to planktonic bacterial surface

The attachment of aqu-nC\textsubscript{60} to different bacterial surface at different equilibrium aqu-nC\textsubscript{60} concentration is shown in Figure 6.5. As the concentration of aqu-nC\textsubscript{60} increased, more fullerene nanoparticles adhered to the bacterial surface; and the bacterial surface was seemingly saturated by aqu-nC\textsubscript{60} when the concentration was larger than 10 mg/L. Obvious differences in attachment efficiency were observed for different bacterial surface. At the same equilibrium concentration, more aqu-nC\textsubscript{60} (per unit area of bacterial surface) were associated with PA, followed by BC, EPS-extracted
BC and PA. The surface charge (Table C3) alone could not explain the difference in attachment of aqu-nC₆₀ to these bacterial surfaces, for example, BC had more nanoparticles adhering to their surfaces than PA despite having less negative surface potential. However, the attachment of aqu-nC₆₀ correlated well with the hydrophobicity of different bacterial surface. The removal of EPS from the bacterial surface not only decreased the surface hydrophobicity (Table C3) that was largely due to the presence of extracellular proteins aforementioned, but also reduced the affinity between hydrophobic nanoparticles like aqu-nC₆₀ with the bacterial surface. EPS characterization (Table C3) also confirmed that there were fewer proteins outside BC surface compared to PA; thus, with EPS, PA was more hydrophobic than BC, which explained why more aqu-nC₆₀ attached to the surface of PA.

![Figure 6.5: Attachment of aqu-nC₆₀ on different bacterial surfaces. Error bars represent ±1 standard deviation.](image)

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The Langmuir adsorption isotherm (Equation 6.1) was used to fit the experimental results shown in Figure 6.5 and the fitted parameters were summarized in Table 6.2.

**Equation 6.1:** \[ q = q_{\text{max}} \frac{KC}{1+KC} \]

where \( q \) (mg/\( \mu \)m\(^2\)) and \( q_{\text{max}} \) (mg/\( \mu \)m\(^2\)) are the mass and maximum mass of attached nanoparticles normalized to the bacterial surface area, respectively, \( C \) (mg/L) is the equilibrium concentration of nanoparticles, and \( K \) (L/mg) is the constant that reflects the affinity of nanoparticles with bacterial surface.

The Langmuir constant \( K \) for EPS-extracted PA was the lowest, probably because they were the least hydrophobic. Approximately 45% of the surface of Gram-negative bacteria is covered by LPS molecules, which are amphiphilic. But the hydrophobic region of LPS, the lipid A, is mainly anchored in the outer membrane; while the hydrophilic O-antigen extends into aqueous solution. Therefore, the surface of Gram-negative bacteria like PA is dominated by hydrophilic region and LPS controls the interaction between Gram-negative bacteria with surfaces, though the hydrophobic interaction is still the major interaction between LPS and hydrophobic surface. Without LPS on its surface, Gram-positive bacteria like BC is more hydrophobic as its outer membrane is more accessible to nanoparticles. The hydrophobic region on the cell membrane and hydrophobic macromolecules such as protein that embedded in the membrane can serve as the hydrophobic sites for hydrophobic nanoparticle to
Table 6.2: Langmuir model parameters for nanoparticles attaching to different bacterial surfaces.

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Bacterial Surface</th>
<th>q&lt;sub&gt;max&lt;/sub&gt; (mg/µm²)</th>
<th>K (L/mg)</th>
<th>R²</th>
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<tbody>
<tr>
<td>Aqu-nC&lt;sub&gt;60&lt;/sub&gt;</td>
<td>EPS-extracted PA</td>
<td>1.64×10&lt;sup&gt;-13&lt;/sup&gt;</td>
<td>1.11</td>
<td>0.9954</td>
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<tr>
<td></td>
<td>EPS-extracted BC PA</td>
<td>2.94×10&lt;sup&gt;-13&lt;/sup&gt;</td>
<td>2.54</td>
<td>0.9928</td>
</tr>
<tr>
<td></td>
<td>PA</td>
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<td>4.60</td>
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<tr>
<td></td>
<td>BC</td>
<td>5.30×10&lt;sup&gt;-13&lt;/sup&gt;</td>
<td>4.66</td>
<td>0.9965</td>
</tr>
<tr>
<td>Fullerol</td>
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<td>0.54</td>
<td>0.9967</td>
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<td>EPS-extracted BC PA</td>
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<td>0.9908</td>
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<td>0.9956</td>
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<td>2.33×10&lt;sup&gt;-13&lt;/sup&gt;</td>
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<td>Ag-CIT</td>
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<tr>
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<td>1.67×10&lt;sup&gt;-12&lt;/sup&gt;</td>
<td>1.35</td>
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<td>Au-CIT</td>
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<td>0.9926</td>
</tr>
<tr>
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<td>Ag-PVP</td>
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<td>0.25</td>
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<td>0.9941</td>
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<td>Ag-GA</td>
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<td>0.20</td>
<td>0.9927</td>
</tr>
<tr>
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<td>0.9811</td>
</tr>
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<td>1.44</td>
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</tbody>
</table>

attach to. The hydrophobic interaction between aqu-nC<sub>60</sub> and BC surface was thus stronger, which was shown by the higher K. The presence of EPS minimized the influence of LPS on the interaction between nanoparticles and bacterial surface and the direct contact of nanoparticles with cell wall. Thus the attachment of nanoparticles to
EPS-coated surface was largely controlled by the interaction between EPS and nanoparticles. The Langmuir constant $K$ was similar for both PA and BC; while $q_{\text{max}}$ for PA was larger than BC. A possible explanation for this was that aqu-nC$_{60}$ attached both to bacterial surface by association with the hydrophobic proteins in the EPS surrounding, thus the similar $K$. However, there were more proteins in the EPS produced by PA than BC, which led to more attachment of nanoparticles per unit area of bacterial surface ($q_{\text{max}}$).

In contrast, the attachment of fullerol to bacterial surface showed no correlation with the hydrophobicity (Figure 6.6). EPS generally increased the total surface charge of bacterial surface, yet enhanced the association between fullerol and bacteria, which indicated that EDL interaction was not the controlling factor. The association of fullerol with large organic macromolecules in the EPS matrix such as polysaccharides$^{2,358}$ might contribute to the higher affinity between fullerol and bacteria in the presence of EPS. In absence of LPS, more fullerol attached to the EPS-extracted BC surface due to less steric interaction, compared to EPS-extracted PA surface.

Similar trends were observed for the attachment of Ag-CIT and Au-CIT to bacteria (Table 6.2 and Figure C4, C5), as BC with the most nanoparticles adhered to them and EPS-extracted PA with the least. The thin-section TEM results of Au-CIT attaching to bacteria were shown in Figure 6.7. No Au-CIT nanoparticles were found on the EPS-extracted PA surface, while a few of them were in very close contact with the
Figure 6.6: Attachment of fullerol to different bacterial surfaces.
Error bars represent ±1 standard deviation.

cell wall of EPS-extracted BC. From Figure 6.7, bacterial surfaces with EPS surrounding the cell wall were evidently more favorable for Au-CIT to adhere to than without EPS. Most nanoparticles associated with PA and BC were not in direct contact with the cell, which indicated that it was the extracellular molecules that nanoparticles attached to, rather than the cell wall. Similar results were observed for the association of Ag-CIT with bacteria.

The interaction of Ag-PVP, a relatively hydrophobic silver nanoparticle resulting from the PVP coatings, with bacterial surface was shown to follow a similar trend as aqu-nC₆₀ (Figure 6.8). Because of the hydrophobic interaction between hydrophobic region of bacterial surface and Ag-PVP, the highest amount of nanoparticles were found on PA surface, the most hydrophobic of all bacterial surfaces investigated. And almost no Ag-PVP attached to the least hydrophobic surface, the EPS-extracted PA. Though steric interaction might limit direct attachment of Ag-PVP to the organic
Figure 6.7: Au-CIT attached to different bacterial surfaces as observed by thin-section TEM.

(a) EPS-extracted PA; (b) EPS-extracted BC; (c) PA and (d) BC.
macromolecules-rich bacterial surface, the long-range hydrophobic interaction was still strong enough to stabilize the adhesion of nanoparticles surrounding the surface. Thin-section TEM results confirmed the configuration of Ag-PVP nanoparticles around bacteria (Figure 6.9). From Figure 6.9, Ag-PVP were clearly not attached to the cell wall, while still managed to stay closely to the bacterial surface.

![Graph showing attachment of Ag-PVP to different bacterial surfaces.](image)

**Figure 6.8: Attachment of Ag-PVP to different bacterial surfaces.** Error bars represent ±1 standard deviation.

However, the attachment of Ag-GA, a similar hydrophobic silver nanoparticle with polymeric surface coatings, to the bacterial surface was minimal (Figure C6). A possible explanation for this observation was that the thickness of GA coatings layer was much larger than that of PVP coatings layer\(^{318}\), which exceeded the effective range of hydrophobic interaction. In this case, the steric interaction dominated the interactions between Ag-GA and bacteria, therefore hindering the attachment of nanoparticles.
Figure 6.9: Ag-PVP attached to different bacterial surfaces as observed by thin-section TEM.
(a) EPS-extracted PA; (b) EPS-extracted BC; (c) PA and (d) BC.
The attachment kinetics of nanoparticles to planktonic bacterial surface was determined on PA surface and the results were shown in Figure 6.10. For all the nanoparticles tested, the attachment to bacteria reached equilibrium after 25 min.

Figure 6.10: Attachment kinetics of different nanoparticles on PA bacterial surface.

It should be pointed out that at the early stage, \( \ln\left(\frac{N}{N_0}\right) \) decreased linearly over time (Figure C7); thereby, attachment efficiency can be calculated based on Equation 6.2 (derivation of Equation 6.2 in Appendix C).

Equation 6.2: \( \alpha = -\frac{S}{\beta B} \)

where \( S \) is the slope of the \( \ln(N/N_0) \sim t \) curve at the early stage, \( \beta \) is the collision efficiency considering diffusion only and using Smoluchowski’s classical model\(^{359}\), and \( N, N_0 \) and \( B \) are the number concentrations of unattached nanoparticles, initial nanoparticles and bacteria, respectively.
Figure 6.11 summarizes the attachment efficiencies calculated from Equation 6.2 for different nanoparticles on PA bacterial surface. Except for aqu-nC₆₀, more hydrophobic nanoparticles achieved greater $\alpha$. However, it should be noticed that the calculation of $\beta$ might oversimplify the real hydrodynamic condition; thus the use of the values of $\alpha$ reported here should be for the purpose of comparison only.

![Bar chart comparing attachment efficiency of different nanoparticles on PA bacterial surface.](image)

**Figure 6.11: Comparison of attachment efficiency for different nanoparticles on PA bacterial surface.**

### 6.7 Effect of pH and IS on the attachment of NPs to bacterial surface

The effect of pH on the attachment of aqu-nC₆₀ to different bacterial surface was shown in Figure 6.12. As the pH changed from basic (pH=9) to acidic (pH=5), aqu-nC₆₀ became more adhesive to any bacterial surface. Since the net surface charge decreased at the same time, it was believed that the reduced EDL interaction was responsible for this enhanced attachment. Similar trends were also observed for fullerol (Figure C8).
Figure 6.12: Effect of pH on the attachment of aqu-nC$_{60}$ to different bacterial surface at the initial concentration of aqu-nC$_{60}$ = 6.3 mg/L.

Error bars represent ±1 standard deviation.

The attachment of Ag-CIT to bacteria at different IS was investigated (Figure 6.13). It was shown in Figure 6.13 that the increasing IS promoted the adhesion of Ag-CIT to any bacterial surface studied. The decreasing zeta-potential of both bacteria and nanoparticles suggested that the compression of EDL by higher IS and thus reduced EDL interaction was the possible reason for more attachment of Ag-CIT to bacteria. No significant aggregation were observed for either bacteria or nanoparticles in a control experiment, which was expected since the highest IS used, 50 mM of NaCl, was below the critical concentration of coagulation (CCC) reported elsewhere $^{360}$.

In contrast, the effect of IS on the attachment of Ag-PVP to bacteria was less pronounced. The increase of IS had minimal effect on the surface charge of Ag-PVP due to the presence of PVP polymer layer. Though surface charge of bacterial surface...
decreased as a result of the increasing IS, the attachment of Ag-PVP to the bacteria remained unchanged (Figure C9), which probably resulted from the steric interaction.

Figure 6.13: Effect of IS on the attachment of Ag-CIT to different bacterial surfaces at the initial concentration of Ag-CIT = 10 mg/L. Error bars represent ±1 standard deviation.

6.8 Summary of results

Figure 6.14 summarizes the relationship between the attachment efficiencies of nanoparticles and the hydrophobicity of different collector surfaces. The presence of a biofilm was generally, but not always observed to increase the affinity of nanoparticles for a porous medium. For hydrophobic nanoparticles such as aqu-nC₆₀ and Ag-PVP, the relative amounts of protein and polysaccharide in the biofilm appear to be important predictors of the degree of affinity for the porous medium with a biofilm, with simple coatings of these two materials bracketing the observed values for the affinity.
coefficients. But this hydrophobic effect is less pronounced for hydrophilic nanoparticles such as fullerol and Ag-CIT.

Figure 6.14: Attachment efficiencies of nanoparticles to collector surfaces with different hydrophobicities. Error bars represent ±1 standard deviation.

In Figure 6.15, the attachment efficiencies of nanoparticles with different hydrophobicities were shown. In general, nanoparticles that are more hydrophobic exhibited a higher affinity for the biofilms and hydrophobic macromolecules-coated collector surface, than hydrophilic nanoparticles. Though for hydrophobic nanoparticles that were sterically stabilized by uncharged macromolecules (i.e. Ag-PVP), the effect of hydrophobic interaction was attenuated by steric interaction.

The presence of divalent ions such as calcium further increased nanoparticle attached to biofilm, protein, and polysaccharide-coated media, with again the exception of nanoparticles that were sterically stabilized. Overall, the hydrophobicity of both the
nanoparticles and the bacterial surfaces are important in determining the intensity of hydrophobic interaction and influencing the affinity between them.

Figure 6.15: Attachment efficiencies of nanoparticles of different hydrophobicities to biofilm or macromolecule-coated collector surfaces. Error bars represent ±1 standard deviation.

The studies on the attachment of nanoparticles to the planktonic bacterial surface further confirmed how hydrophobicity mediates the interaction between nanoparticles and the bacterial surface. The presence of EPS not only made the surface more charged, but also brought about greater hydrophobicity that significantly enhance the affinity for nanoparticles. The change of environmental conditions such as pH and ionic strength affected the attachment of nanoparticles by altering the surface charge, which suggested that the influence of DLVO interactions should not be neglected.

In the present study, two typical yet distinct kinds of bacterial surface (i.e. gram-negative and gram-positive) were selected to represent those bacterial surfaces that environment engineered nanoparticles might encounter in aqueous systems. However,
the enormous number of species of bacteria, the various phases of biofilm growth as well as different environmental condition might further complicate the scenarios investigated here. The effect of divalent ions, pH and ionic strength on the surface chemistry of bacteria and the attachment of nanoparticles to them were also good examples of the challenge we face in better understanding the fate an transport of engineering nanoparticles in biofilm-laden porous media and furthermore, the natural aquatic environment.
7 Conclusions

This dissertation research investigated three core questions focusing on the characterization and implication of surface hydrophobicity in the fate and transport of nanoparticles. In this chapter, conclusions to each of the three questions are summarized, and implications are discussed.

7.1 Can the distribution of nanoparticles in an immiscible water-oil system be used to predict hydrophobicity?

7.1.1 Hypotheses and objectives

The hypothesis was tested that surface hydrophobicity affects the distribution of nanoparticles in a water-oil system and that nanoparticles accumulating at the liquid-liquid interface are large amphiphilic particles.

Calculation using multiple thermodynamic models with different treatment of interfacial energy was explored, and theoretical prediction was proposed. This was examined against experimental studies in a water-octanol system, considering the influence of a variety of factors, including hydrophobicity, on the distribution behavior of nanoparticles, with an emphasis on how they behave at the water-octanol interface.

7.1.2 Summary of evidence

The thermodynamic calculations based on solid-liquid interfacial energy suggested both size and hydrophobicity controlled the distribution of nanoparticles between water and octanol, and as the particle surface became amphiphilic or particle
size increased up to 1 – 10 nm, an enormous energy well would trap the particle at the water-octanol interface. Meanwhile, another thermodynamic calculation involving cavity formation, without using solid-liquid interfacial energy that is often not accessible, was carried out. It was shown that, in addition to hydrophobicity and particle size, surface charge also influenced the distribution and attenuated the energy well at the interface by providing a repulsive electrostatic interaction.

As for the experimental studies, hydrophobic nanoparticles (e.g. aqu-nC₆₀) generally preferred to stay in the octanol, and the neutralized surface charge and increased size, either by pH adjustment towards their isoelectric point, increasing ionic strength or successive filtration, led to more hydrophobic nanoparticles in octanol. However, the favorable phase for hydrophilic nanoparticles (e.g. fullerol) was water and the change of surface charge did not affect their dominant presence in aqueous phase. For Ag-CIT and Au-CIT, despite moderately favoring water, as the surface charge diminished and size increased as a result, more of them accumulated at the water-octanol interface. More of larger Ag-PVP (~ 40 nm) stayed at the water-octanol interface than smaller Ag-PVP (~ 10 nm).

### 7.1.3 Conclusions

The hydrophobicity of nanoparticles as measured by several methods evaluated in this work was a good predictor for octanol-water partitioning, while particle size and surface charge also affected their distribution. Diameters of 1 to 10 nm represented a
threshold, beyond which amphiphilic nanoparticles would congregate at the water-octanol interface, though the presence of large surface charge might prevent the accumulation of nanoparticle there.

7.2 What are suitable methods for characterizing surface hydrophobicity of nanoparticles?

7.2.1 Hypotheses and objectives

Chapter 5 tested the hypothesis that the characterizations of hydrophobicity by macro-scale, solute-scale and nano-scale adsorptive methods are consistent. In addition, the hypothesis that water-affinity based methods applied to nanomaterial powders can yield a thermodynamic interpretation of hydrophobicity was also examined.

Evaluating different characterization techniques for hydrophobicity at different size-scale and identifying suitable method for our intended research was the primary objective for examining this hypothesis. These methods were assessed by their consistency and how to interpret measurement result.

7.2.2 Summary of evidence

The surface hydrophobicity of six different NPs, which included carbon-based fullerene and fullerol, and silver/gold-based without (Ag-CIT and Au-CIT) and with polymeric coatings (Ag-PVP, Ag-GA), were evaluated by a number of characterization methods.

Smaller contact angle was observed on a thin film of more hydrophilic NPs, while larger contact angle on less hydrophilic ones. Larger $K_{ow}$ were measured for more
hydrophobic NPs, though congregation of NPs at the water-octanol interface was observed and might interfere the $K_{ow}$ measurement. More RB and naphthalene, selected hydrophobic chemical probes, were adsorbed on hydrophobic NP surface (e.g. fullerene, Ag-PVP), and there was no adsorption of these probes on hydrophilic particles (e.g. fullerol, Ag-CIT). Other interactions between hydrophobic probes and NPs were found to possibly compromise the reliability of this method, such as the electrostatic interaction for RB and $\pi-\pi$ interaction for naphthalene.

Except for fullerene, a monolayer of water vapor formed on the surface of nanoparticle powders in the dynamic water vapor adsorption experiment. After fitting the adsorption isotherm to BET equation, BET constants were obtained and found to correlate with the hydrophobicity of nanoparticles obtained by previous discussed methods. However, the decreasing of surface area likely occurred during the adsorption, brought about by dissolution of nanoparticles or desorption of coatings. As a result of aggregation states changing or hydration of residual polymers, the immersion microcalorimetry experiments yielded contradictory results and the measured immersion enthalpy was not considered as a good indicator of the hydrophobicity. In TGA experiments, the weight loss of nanoparticle powders between 120°C and 500°C in nitrogen was quantified and shown to correlate well with the hydrophobicity, as there were less chemically adsorbed water and functional groups that had strong affinity for water on more hydrophobic surfaces.
7.2.3 Conclusions

The methods evaluated provide a largely consistent description of the surface hydrophobicity of nanoparticles and a qualitative ranking of their relative hydrophobicity, rather than quantitative measurement of hydrophobicity. The adsorption of molecular probes method produced consistent results with most of other methods in terms of the order of hydrophobicity for the nanoparticles tested, despite that each of those methods had some limitations. Compared to other in-situ characterization methods, adsorptive method offers more solid grounds for interpretation of the results. Admittedly, some water-affinity based characterization methods that applied to nanomaterial powders yielded consistent results with those in-situ measurements. However, considering the still unclear physical or chemical transformations to the surface area and surface properties during the characterization of nanomaterial powders, a rigorous thermodynamic model cannot be used to interpret the results from these water-affinity based methods and to infer hydrophobicity. Thus, in-situ characterization provided a more accurate description of hydrophobicity for nanoparticles in most contexts of research interest.

7.3 Can the attachment of nanoparticles to bacterial surface be predicted by hydrophobic interaction?

7.3.1 Hypotheses and objectives

In Chapter 6, the third hypothesis that hydrophobic interaction enhances the attachment of hydrophobic nanoparticles to the hydrophobic bacterial surface was
examined on planktonic and biofilm surface of Gram-positive and Gram-negative bacteria. Column experiments were conducted to calculate the attachment efficiency, which can quantify the affinity of nanoparticles for the biofilm. Batch attachment experiments were also carried out for investigating the interaction between nanoparticles and planktonic surface in the presence and absence of EPS.

**7.3.2 Summary of evidence**

We reported out findings regarding the role of hydrophobic interaction in the attachment of nanoparticles to bacterial surfaces of different surface components and most importantly, different surface hydrophobicity, in Chapter 6.

The attachment efficiencies of aqu-nC₆₀, fullerol, Ag-CIT and Ag-PVP were obtained from the column deposition experiments, using glass beads coated with Gram-negative PA and Gram-positive BC biofilm to simulate the biofilm-laden porous media. The hydrophobicity of biofilm was found to correlate with the relative quantity of proteins and polysaccharide in EPS; therefore, the deposition of nanoparticles on BSA and alginate-coated glass beads were also evaluated. The attachment efficiencies of hydrophobic nanoparticles (i.e. aqu-n C₆₀) on PA and BC biofilm were bracketed by those on BSA and alginate-coated surfaces, and the affinity of NPs was stronger for PA biofilm with a larger proteins/polysaccharides ratio compared to BC biofilm. The presence of biofilm retarded the mobility of nanoparticles without polymeric coatings (i.e. aqu-n C₆₀, fullerol and Ag-CIT), but it had little effect on the retention of Ag-PVP,
which might be because of the steric interaction. The pre-treating by Ca\(^{2+}\) significantly
hydrophobized biofilm, BSA and alginate-coated glass beads, and the attachment
efficiencies of aqu-nC60, fullerol and Ag-CIT were increased. However, it was again not
the case for Ag-PVP.

The attachment of nanoparticles to planktonic PA and BC surface with and
without EPS was investigated by batch attachment experiment. The formation of EPS
resulted in more surface charge and more hydrophobic bacterial surface as shown by the
characterization of bacterial surface. PA had the most hydrophobic surface, followed by
BC, EPS-extracted BC and PA. The amount of hydrophobic aqu- C60 adsorbed on the
bacterial surface followed the same order, with least C60 found on least hydrophobic
EPS-extracted PA surface because of LPS. Similar trend was observed for another
hydrophobic nanoparticle, Ag-PVP. However, for fullerol, Ag-CIT and Au-CIT, the
effect of hydrophobic interaction was not evident, though EPS generally enhanced the
affinity of bacterial surface for nanoparticles. By adjusting pH towards the isoelectric
point or increasing the ionic strength, the attachment of aqu-n C60, fullerol, Ag-CIT and
Au-CIT to bacteria were all improved, suggesting that the weakened electrostatic
repulsion could affect the attachment.

**7.3.3 Conclusions**

In conclusion, when the bacterial surface, planktonic or biofilm, was rendered
hydrophobic by the components of EPS, namely proteins, its affinity for hydrophobic
nanoparticles was controlled by hydrophobic interaction. The attachment of nanoparticles with more hydrophobic surface to biofilm was generally greater than less hydrophobic or hydrophilic ones. However, the repulsive steric interaction between polymeric coatings and biofilm might reduce the affinity of hydrophobic nanoparticles for biofilm. The attachment of hydrophobic nanoparticles was also influenced by the hydrophobicity of bacteria surface; while for hydrophilic NPs, this relationship was not observed. Thus, the hydrophobicity of both nanoparticles and bacterial surfaces are important in understanding the hydrophobic interactions and the affinity between them.

7.4 Implications and future research

Considering the lack of a universally accepted explanation to the origin of hydrophobicity and hydrophobic interaction, the intention of this research is not to explore the underlying mechanism for the phenomena related to the hydrophobicity of nanoparticles, but rather to extend our knowledge regarding hydrophobic interaction at the molecular level and the macroscopic scale to nanoparticles, which sits at the transition region between these two. This dissertation is the first systematic attempt to evaluate the role of hydrophobicity in the fate and transport study of nanomaterial. While it has filled some knowledge blanks, several implications arise and are summarized below.
7.4.1 Characterization strategy for surface hydrophobicity of nanoparticles

Thorough characterization of physical and chemical properties of NPs is fundamental in the investigation of potential exposure, hazard and hence risk of nanomaterial in the environment. Without a descriptive assessment, we are unable to repeat research and to analyze results within the universe of other nanoparticle studies. Nanoparticles are not solutes, the characterization of which usually means exposure concentration. Many physicochemical properties of nanoparticles have been characterized such as size distribution, aggregation status, shape, surface area, surface chemistry, surface reactivity and crystallinity. There are so many properties that two questions are often times asked: 1) What properties are the key properties that are crucial to the environmental and ecotoxicological studies of nanomaterial? 2) What properties can be used to group different nanomaterials into categories that are considered to behave similarly in environmental studies? Among those frequently mentioned as key properties, surface chemistry represents a significant one, as it affects how nanoparticles interact with their surroundings; yet its definition is rather vague, and difficult to quantify. This work has established a relatively convenient measure of surface hydrophobicity, and demonstrated its important role in the fate and transport of nanoparticles. Thereby, surface hydrophobicity can serve as a semi-quantifiable parameter of surface chemistry, and it should be included as one of the key properties of the overall characterization strategy for nanoparticles. Moreover, it is shown in Chapter
4 and 5 that, in spite of different core material, nanoparticles with similar 
hydrophobicity behaved alike at both liquid-liquid and liquid-solid interfaces. This 
phenomenon suggests the potential use of hydrophobicity in dividing nanoparticles into 
different groups for relevant environmental studies.

In developing a comprehensive and effective characterization strategy, another 
crucial question that needs to be addressed is: when to characterize? Do we focus on the 
initial material characterization of the static properties of nanomaterial as is prepared, or 
emphasize on the fate characterization of the dynamic properties that are everchanging? 
The former strategy exerts no real analytical constraints as nanomaterial can be 
distributed around to be characterized by expert labs, and simplifies the input for a risk 
assessment model by providing a value that applies to the life cycle of the nanomaterial. 
In contrast, the latter depends on an agreement on the dispersion and application 
protocol of nanomaterial due to the constantly changing of properties under various 
environmental conditions, or a case-by-case characterization strategy is obliged. 
However, it yields a realistic description of status of the nanomaterial in the scenario we 
are interested in. Take surface hydrophobicity as an example, the as-prepared and in-

situ characterization methods employed by this dissertation correspond to these two 
strategies. Inconsistent results were obtained between these two kinds of methods. The 
dispersion of nanoparticles in the aqueous medium, either by modifying the surface (e.g. 
hydroxylation of fullerene) or adding stabilizing agents (e.g. polymeric coatings on Ag-
NPs), inevitably brings in uncertainties to the surface hydrophobicity. The environmental transformations such as oxidative dissolution\textsuperscript{361} or oxysulfidation\textsuperscript{362} further alter the surface chemistry of nanoparticles. Thus, we face a dilemma of which strategy to choose and it should be dependent on our research needs.

The method validation and harmonization is equally important as method development in establishing a characterization strategy. A spectrum of as-prepared and in-situ characterization methods for hydrophobicity was evaluated in Chapter 2, and the advantages and disadvantages were discussed. To assure the measurement quality, these methods need to be optimized. For the surface adsorption of hydrophobic chemical probes method, future research is needed in search of an ideal probe molecule or a combination of probes that can exclude the interferences of other interactions to the adsorption process and enlarge the range of hydrophobicities this method can measure. A different organic solvent than octanol to perform the partitioning experiment that can better fractionate nanoparticles by hydrophobicity and minimize emulsion at the interface will greatly expand the application of partition coefficient. The measurement of contact angle at the nano-scale by utilizing more sensitive techniques with larger magnification\textsuperscript{151,363} and will reveal more details on the hydrophobicity of individual nanoparticle, rather than an average measurement on a thin film of nanoparticles. For method harmonization, standard reference nanomaterials of different hydrophobicity should be identified for each method. For instance, carboxylated and hydroxylated
polystyrene particles were used as the standard reference for the RB adsorption method. In addition, quality assurance and quality control need to be performed for these methods.

7.4.2 What does hydrophobicity tell us?

Having extensively studied hydrophobicity, hydrophobic interaction and nanoparticles, naturally we are curious to know: what does hydrophobicity tell us in terms of the environmental implication of nanomaterial? What information about the environmental risks of nanomaterial can we infer from surface hydrophobicity? The implication of hydrophobicity is two-fold: mobility and bioavailability, and it will be discussed from these two perspectives.

7.4.2.1 Implication of hydrophobicity for the fate and transport study of nanoparticles

In Chapter 4 and 5, water-octanol interface and water-bacteria interface were selected as two example scenarios to investigate how surface hydrophobicity and hydrophobic reaction affected the fate and transport of nanoparticles in aquatic environment, and it was shown that the influence of hydrophobicity affects many aspects of nanoparticle fate.

First, hydrophobic nanoparticles readily aggregate in aqueous medium, and their size grows quickly so that once aggregates become large enough, settling may occur. This phenomenon has been observed, when aqu-nC_{60} was prepared by extended mixing, that large chunks of C_{60} always form and settle after a period of time. Thus, special
treatments are usually needed to disperse hydrophobic nanoparticle powders, such as making the surface less hydrophobic by functional groups (e.g. hydroxyl and carboxyl groups) or using surfactants and polymers to stabilize them from aggregation. However, these coatings might be removed from the surface due to desorption or dissolution in some cases, and such dispersed hydrophobic nanoparticles are still more likely to aggregate and become less mobile in the aquatic environment. On the contrary, hydrophilic nanoparticles prefer to remain dispersed in water by so-called “hydrophilic interaction” \(^{32}\). Thus their potential to be left out of aqueous medium by aggregation and sedimentation is much lower.

Second, as illustrated in Chapter 5, the affinity of hydrophobic nanoparticles for the hydrophobic region on the water-solid interface was very strong as a result of hydrophobic interaction, and the mobility of nanoparticles in the presence of hydrophobic surface was greatly retarded. There is no shortage of such surfaces in the natural and engineered environment, for example, the biofilm, the soil organic matter, the filter media \(^{318}\), and the activated sludge in the wastewater treatment plant. Those hydrophobic surfaces can all serve as environmental sinks for hydrophobic nanoparticles. Yet for hydrophilic nanoparticles, there is no existence of such preferentially sinks in the natural environment.

Third, a main conclusion of Chapter 4 is that the large amphiphilic nanoparticles tend to accumulate at the liquid-liquid interface. As discussed earlier, the surface of
most initially hydrophobic nanoparticles are modified to closer to amphiphilic in order to be dispersed in water. Thus, based on our research, the most likely environmental destinations for those nanoparticles are those liquid-liquid interfaces in aquatic environment such as DNAPL zone, lipid-rich sea-surface microlayer, and biofilm.

Finally, hydrophobic interaction can lead to the attachment of hydrophobic nanoparticles to many environmental colloids such as planktonic bacteria, and the adsorption of dissolved hydrophobic organic contaminants on nanoparticles. This piggybacking effect may alter the transport behavior and mobility of any of these species.

7.4.2.2 Is hydrophobicity a predictor bioavailability?

From the perspective of tradition risk assessment, hydrophobicity is a very reliable predictor of bioavailability for small molecules like organic compounds. This prediction holds, because small molecules can cross the cellular membrane of an organism from the environment. This movement can be accomplished by passive diffusion and driven by hydrophobic interaction. Similarly, the partitioning of small molecules between water and a hydrophobic solvent is often considered as a good indicator of hydrophobicity, based on the assumption that molecules can move freely between each phase and stay at the phase they have stronger affinity for. Thus, traditionally the partition coefficient, hydrophobicity and bioavailability are often used interchangeably for small molecules. Then the question arises: does this relationship
apply for nanoparticles? Intuitively, many researchers reject this relationship for nanoparticles, as they believe that the larger size of nanoparticles becomes an obstacle for them to move freely either from one liquid phase to the other or from environment to the inside of cells. However, the answer to this question is believed to be more complicated than a simple “No”.

We tentatively investigated the relationship between partitioning and hydrophobicity in Chapter 4. First of all, it was shown that accumulation at the liquid-liquid interface is not an automatic phenomenon for nanoparticles. Thermodynamic calculation predicted that 1 – 10 nm represents a size threshold beyond which the majority of nanoparticles will be trapped at the liquid-liquid interface. Experimental studies confirmed this prediction, and suggested that due to the presence of surface charge or polymeric coatings that provide a repulsive force between particles at the interface, this size threshold might be even larger. But an amphiphilic surface would expedite the accumulation of nanoparticles at the liquid-liquid interface. Secondly, neglecting the particles at the interface, the distribution of nanoparticles between octanol and water still gave a consistent estimation of the hydrophobicity. In fact, if the portion of nanoparticles at the interface is not large, which was the case for most of our experiments, and partition coefficient was calculated based on the mass rather than, traditionally, the concentration of nanoparticles, the partition coefficient appears to be a serviceable indicator for hydrophobicity compared with other indicators.
As for the relationship between hydrophobicity and bioavailability, it has been a well-established knowledge that, due to the presence of cell wall and the larger size of nanoparticles compared to small molecules, it is virtually not possible for nanoparticles to get into bacteria cells by partitioning. Thus, the fundamental assumption to predict bioavailability using hydrophobicity appears to have been compromised. However, as shown in Chapter 5, the attachment of hydrophobic nanoparticles to the bacterial surface, planktonic or attached (i.e. biofilm), with or without EPS, was greater than hydrophilic ones. This observation suggests that hydrophobicity can be an indicator of the bioaccessibility of nanoparticles. The attachment of nanoparticles at the bacterial surface might penetrate or disrupt the cell membrane. The dissolution of nanoparticles in close proximity to bacteria might lead to greater toxicity. Admittedly, bioaccessibility is not exactly the same as bioavailability, but it is related to bioavailability. When nanoparticles become more accessible to bacteria, the risk they pose to bacterial might be different compared to when they are further apart. Future research is definitely needed on this problem.

### 7.4.3 Applications of hydrophobicity and hydrophobic interaction

The prevailing theme throughout this dissertation was the role of hydrophobicity in the fate and transport of nanoparticles in the natural environment. However, the knowledge gained from this study will significantly improve our understanding of how to utilized hydrophobicity and hydrophobic interaction in environmental applications.
The in situ remediation of NAPL source zones using polymer-modified nanoscale zerovalent iron (NZVI) particles has been extensively investigated as an effective remediation technique. The mobility of NZVI in porous media and the targeted delivery of NZVI to the NAPL/water interface greatly affect the efficiency of remediation. The results from Chapter 5 and 4 will contribute toward better design of NZVI particles and polymer-coatings to maximize the mobility of NZVI and improve the targeting accuracy. In addition, the delivery of NZVI and other remediation agents like engineered bacteria can be accomplished by a micelle system such as liposome.

Our research on the behavior of nanoparticles at the liquid-liquid interface has important implication on the material fabrication based on self-assembly. The results obtained from the effect of hydrophobicity on the attachment of nanoparticles to bacterial surface can help further studies in designing anti-biofouling membrane materials. Moreover, continuous research on hydrophobicity and hydrophobic interaction will facilitate the development of novel self-cleaning material and drug delivery vehicles.
Appendix A. Supporting information for Chapter 4

Figure A 1: Contact angle at solid-water-oil interface.

Figure A 2: Energy well at the interface for nanoparticles with different size and contact angle.
Figure A 3: Energy well at the interface of amphiphilic nanoparticles with different size.

Table A 1: Physical parameters of solvents and cavity formation energy of particle with different sizes at 25°C.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\sigma_1 \times 10^{10}, \text{m}$</th>
<th>$\rho \times 10^{27}, \text{m}^{-3}$</th>
<th>$y$</th>
<th>$\Delta G_c, \text{kJ} \cdot \text{mol}^{-1}$</th>
<th>$\sigma_2 = 1 \text{ nm}$</th>
<th>$\sigma_2 = 10 \text{ nm}$</th>
<th>$\sigma_2 = 100 \text{ nm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>2.75</td>
<td>33.3</td>
<td>0.36</td>
<td>120</td>
<td>1.05x10^4</td>
<td>1.07x10^6</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>5.26</td>
<td>6.76</td>
<td>0.52</td>
<td>90.8</td>
<td>7.56x10^3</td>
<td>7.48x10^5</td>
<td></td>
</tr>
<tr>
<td>Heptane</td>
<td>5.996</td>
<td>4.08</td>
<td>0.46</td>
<td>52.6</td>
<td>4.16x10^3</td>
<td>4.10x10^5</td>
<td></td>
</tr>
<tr>
<td>Octanol</td>
<td>6.55</td>
<td>3.80</td>
<td>0.56</td>
<td>80.2</td>
<td>6.52x10^3</td>
<td>6.42x10^5</td>
<td></td>
</tr>
</tbody>
</table>
Figure A 4: Change of cavity formation energy normalized by surface area of solute particle as particle size changes.

Derivation of the van der Waals interaction energy between a solute particle and solvent (Equation 4.9)

Suppose the energy of the van der Waals interaction between a solute molecule and a given solvent molecule with a distance $D$ away is $\epsilon_{12}(D)$.

Equation A 1: $\epsilon_{12}(D) = -\frac{C_{12}}{D^6}$

where $C_{12}$ (1 – solvent; 2 – solute) is the coefficient that sums up contributions of all three major components of the van der Waals interaction: Keesom, Deby, and London dispersion interaction $^{35}$.

Since the sum of $\epsilon_{12}$ over the whole solvent, $\tilde{E}_i$, is approximately equal to $\tilde{H}_i$ because of the fact that the solvent is a condensed phase, the van der Waals interaction
energy between a solute molecule and the solvent $\tilde{G}_{i,m}$ can be determined by integration of the Gibbs-Helmholtz relationship. Thus, $\tilde{G}_{i,m}$ can be calculated by $^{121}$,

**Equation A 2:** $\tilde{G}_{i,m} \equiv E_i = N \int_{vol} \epsilon_{12}(D) \times 4 \pi D^2 \rho_1 g(\sigma_2, D) dD$

where $\rho_1$ is the number density of solvent, $N$ is the Avogadro’s number, and $g(\sigma_2, D)$ is a radial distribution of finding a solvent molecular center at the distance $D$ from the center of a solute molecule of radius $\sigma_2$, which can be assumed to be unity as an approximation $^{121}$.

When considering a molecule inside a particle that is immersed in the solvent, Equation A2 must be adapted to calculate $\tilde{G}_{i,m}$ for this molecule. Suppose the molecule is located at a distance of $r$ away from the center of the particle (radius = $R$), then the integration can be evaluated in three parts. When $0 \leq D < R - r + \sigma_{12}$ ($\sigma_{12} = (\sigma_1 + \sigma_2)/2$), the integration is zero, as there is no solvent molecules in this region (i.e. $g(\sigma_2, D) = 0$). When $R - r + \sigma_{12} \leq D < R + r + \sigma_{12}$, $g(\sigma_2, D)$ is equal to unity only in the shaded area while 0 elsewhere (Figure A5). Then Equation A2 becomes,

**Equation A 3:** $\tilde{G}_{i,m} = N \int_{R-r+\sigma_{12}}^{R+r+\sigma_{12}} \frac{-L_{12}}{D^6} \times 2 \pi D \frac{(D+r)^2-R^2}{2r} \rho_1 dD$

When $D \geq R + r + \sigma_{12}$, $g(\sigma_2, D)$ is equal to unity everywhere, thus the equation (put equation number here) becomes,

**Equation A 4:** $\tilde{G}_{i,m} = N \int_{R+r+\sigma_{12}}^{\infty} \frac{-L_{12}}{D^6} \times 4 \pi D^2 \rho_1 dD$

Therefore, $\tilde{G}_i$ for the interaction between a solute molecule and the solvent is the integration over all range of $D$. 

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Equation A 5: $$\overline{G}_{i,m} = N \left[ \int_{R-r^+_1}^{R+r^+_1} \rho_1 \left( \frac{(D+r)^2-R^2}{2r} - \rho_1 dD + \int_{\pi R^2}^{\infty} \frac{1}{D_6} \times 4 \pi D^2 \rho_1 dD \right) \right]$$

The integration of $$\overline{G}_{i,m}$$ over the body of the solute particle yields the total energy of interaction $$\overline{G}_i$$

Equation A 6: $$\overline{G}_i = \int_0^R \sigma_{i,m} \times 4\pi^r \rho_2 d\sigma$$

where $$\rho_2$$ is the number density of solute. The integration yields,

Equation A 7: $$\overline{G}_i = \frac{1}{24} A \left[ -\frac{(R^2-Z^2)(4R^3-3R^2Z^2+2RZ^2+Z^3)}{R^3Z^3} + 8 \ln \left( \frac{R}{Z} \right) \right]$$

where $$A$$ is the Hamaker constant, and $$A = \pi^2 \sigma_{12} \rho_1 \rho_2$$.

Figure A 5: Illustration of the van der Waals interaction between a molecule inside the solute particle and solvent.

Table A 2: Cavity formation energy, interaction energy and total solvation energy of different nanoparticles.

<table>
<thead>
<tr>
<th>NPs</th>
<th>$$A \times 10^{20}$$, J</th>
<th>$$\bar{G}_c, kJ mol^{-1}$$ Octanol</th>
<th>Water</th>
<th>$$\bar{G}_i, kJ mol^{-1}$$ Octanol</th>
<th>Water</th>
<th>$$\Delta G, kJ mol^{-1}$$ Octanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqu-nC60</td>
<td>6.48</td>
<td>6.42×10^5</td>
<td>1.07×10^6</td>
<td>-2.08×10^4</td>
<td>-2.76×10^4</td>
<td>-4.21×10^5</td>
<td></td>
</tr>
<tr>
<td>Ag-CIT</td>
<td>9.1</td>
<td>6.52×10^3</td>
<td>4.20×10^4</td>
<td>-5.11×10^3</td>
<td>-1.46×10^4</td>
<td>-2.60×10^4</td>
<td></td>
</tr>
<tr>
<td>Au-CIT</td>
<td>11.83</td>
<td>6.52×10^3</td>
<td>4.20×10^4</td>
<td>-6.42×10^3</td>
<td>-1.94×10^4</td>
<td>-2.25×10^4</td>
<td></td>
</tr>
</tbody>
</table>
Figure B 1: Weight loss versus temperature for fullerene in TGA.
Figure B 2: Weight loss versus temperature for fullerol in TGA.
Figure B 3: Weight loss versus temperature for Ag-PVP in TGA.
Figure B 4: Weight loss versus temperature for Ag-GA in TGA.
Figure B 5: Weight loss versus temperature for Ag-CIT in TGA.
Figure B 6: Weight loss versus temperature for Au-CIT in TGA.
Figure B 7: Weight loss versus temperature for TiO$_2$ in TGA.
Equation B 8: Weight loss versus temperature for TiO$_2$-SiO$_2$ in TGA.
Appendix C. Supporting information for Chapter 6

Figure C 1: Biofilm composition of PA and BC in the column.
Error bars represent ±1 standard deviation.

Table C 1: Composition of synthetic nutrient solution.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>KH$_2$PO$_4$</th>
<th>K$_2$HPO$_4$</th>
<th>KNO$_3$</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1 g/L</td>
<td>1 g/L</td>
<td>1 g/L</td>
<td>1 g/L</td>
</tr>
<tr>
<td>Chemicals</td>
<td>MgSO$_4$</td>
<td>CaCl$_2$</td>
<td>Trace elements*</td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>0.2 g/L</td>
<td>0.02 g/L</td>
<td>1 mL/L</td>
<td></td>
</tr>
</tbody>
</table>

*Trace elements was prepared by adding 33.8 mL of HCl, 7.5 g of FeCl$_3$·4H$_2$O, 0.3 g of H$_2$BO$_3$, 0.5 g of MnCl$_2$·4H$_2$O, 0.6 g of CoCl$_2$·6H$_2$O, 0.35 g of ZnCl$_2$, 0.125 g of NiCl$_2$·6H$_2$O, 0.075 g of CuCl$_2$·2H$_2$O, 0.125 g of NaMoO$_4$·2H$_2$O, and 26 g of EDTA Na$_2$(H$_2$O)$_4$ in 3 L of double-distilled water, filling to 4.8 L, adding NaOH until the pH is 4.2 and then filling to 5 L.

Table C 2: EPM and ζ-potential of porous media with different coatings.

<table>
<thead>
<tr>
<th></th>
<th>Clean-GB</th>
<th>PA biofilm</th>
<th>BC biofilm</th>
<th>BSA</th>
<th>Alginate</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPM$\times 10^{-9}$ m$^2$V$^{-1}$s$^{-1}$</td>
<td>-5.22±0.30</td>
<td>-2.99±0.23</td>
<td>-1.31±0.16</td>
<td>-1.43±0.15</td>
<td>-2.86±0.21</td>
</tr>
<tr>
<td>ζ-potential/mV</td>
<td>-66.58±3.76</td>
<td>-38.12±2.94</td>
<td>-16.73±2.02</td>
<td>-18.27±1.89</td>
<td>-36.48±2.68</td>
</tr>
</tbody>
</table>

*All the values in this table are means ± 95% C.L.
Figure C 2: Relative hydrophobicity of different collector surfaces as measured by adsorption of RB.
Error bars represent ±1 standard deviation.

Figure C 3: Relative hydrophobicity of different collector surfaces after pre-treated by Ca$^{2+}$.
Error bars represent ±1 standard deviation.
Table C 3: Characteristics of different planktonic bacterial surfaces.

<table>
<thead>
<tr>
<th></th>
<th>PA</th>
<th>BC</th>
<th>EPS-extracted PA</th>
<th>EPS-extracted BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPM ($/10^{-8}m^2V^{-1}s^{-1}$)</td>
<td>-2.34±0.28</td>
<td>-1.19±0.16</td>
<td>-1.01±0.14</td>
<td>-0.82±0.15</td>
</tr>
<tr>
<td>ζ-potential/mV</td>
<td>-29.85±3.57</td>
<td>-15.18±2.04</td>
<td>-12.88±1.79</td>
<td>-10.46±1.91</td>
</tr>
<tr>
<td>Hydrophobicity$^b$</td>
<td>0.061</td>
<td>0.049</td>
<td>0.012</td>
<td>0.029</td>
</tr>
</tbody>
</table>

$^a$ All the values in this table are means ± 95% C.L.
$^b$ The hydrophobicity was measured by Rose Bengal adsorption method as described in section 2.2.3

Figure C 4: Attachment of Ag-CIT to different bacterial surfaces.
Error bars represent ±1 standard deviation.
Figure C 5: Attachment of Au-CIT to different bacterial surfaces. Error bars represent ±1 standard deviation.

Figure C 6: Attachment of Ag-GA to different bacterial surfaces. Error bars represent ±1 standard deviation.
Figure C 7: Attachment kinetics of different nanoparticles on PA bacterial surface.

Derivation of the calculation of attachment efficiency from the early-stage kinetics of nanoparticle attaching to planktonic bacterial surface (Equation 6.2)

At the beginning of the batch attachment experiment, the number concentration of nanoparticles and bacteria are $N_0$ and $B$, respectively. Assuming that no homo-aggregation (i.e. aggregation between NP or between bacteria) occurs, once the attachment experiment starts, the number concentration of unattached nanoparticles, $N$, follows,

Equation C 1: \[
\frac{dN}{dt} = -\alpha \beta NBt + k_D (N_0 - N)
\]

where $\alpha$ is the attachment efficiency, $\beta$ is the collision efficiency, and $k_D$ is the constant for detachment of nanoparticles from bacterial surface. At the early stage, it can be assumed that $k_D = 0$. Thus, by integrating Equation C1, it yields,

Equation C 2: \[
\ln\left(\frac{N}{N_0}\right) = -\alpha \beta B t
\]
Then $\alpha$ can be calculated from Equation C2 based on the early-stage of the $\ln(N/N_0) \sim t$ curve following Equation C3,

**Equation C 3:** $\alpha = -\frac{S}{\beta B}$

where $S$ is the slope of $\ln(N/N_0) \sim t$ curve at the early stage.

**Figure C 8:** Effect of pH on the attachment of fullerol to different bacterial surfaces at the initial concentration of fullerol = 10 mg/L. Error bars represent ±1 standard deviation.
Figure C 9: Effect of IS on the attachment of Ag-PVP to different bacterial surfaces at the initial concentration of Ag-PVP = 10 mg/L.
Error bars represent ±1 standard deviation.
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Biography

Yao Xiao was born in 1982 in Chongqing, China. Mr. Xiao obtained a Bachelor and Master of Science in Environmental Science and Engineering at Tsinghua University, Beijing, China (2004 and 2006), followed by a Doctor of Philosophy in Civil and Environmental Engineering at Duke University, Durham, North Carolina (2012).