

# Adsorption of a Cationic Dye Molecule on Polystyrene Microspheres in Colloids: Effect of Surface Charge and Composition Probed by Second Harmonic Generation

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Nonlinear optical probe, second harmonic generation (SHG), of the adsorption of the dye molecule malachite green (MG), in cationic form at  $\text{pH} \leq 5$ , on polystyrene microspheres in aqueous solution is used to study the effect of surface charge and composition on molecular adsorption. Three types of polystyrene microspheres with different surface composition are investigated: (1) a sulfate terminated, anionic surface, (2) a neutral surface without any functional group termination, and (3) an amine terminated, cationic surface. The cationic dye was found to adsorb at all three surfaces, regardless of surface charge. The adsorption free energies,  $\Delta G$ 's, measured for the three surfaces are  $-12.67$ ,  $-12.39$ , and  $-10.46$  kcal/mol, respectively, with the trend as expected from the charge interactions. The adsorption density on the anionic surface, where attractive charge–charge interaction dominates, is determined by the surface negative charge density. The adsorption densities on the neutral and cationic surfaces are on the other hand higher, perhaps as a result of a balance between minimizing repulsive charge interaction and maximizing attractive molecule–substrate and intermolecular interactions. The relative strength of the SH intensity per molecule, in combination of a model calculation, reveals that the  $C_2$  axis of the MG molecule is nearly perpendicular to the surface on the anionic surface and tilts away from the surface norm when the surface is neutral and further away when cationic. Changing the pH of the solution may alter the surface charge and subsequently affect the adsorption configuration and SH intensity.

## 1. Introduction

It has been well established that the properties of the colloidal particle surface are a deterministic factor in defining the properties of a colloid. One of the commonly used practices to modify particle surface properties is to have molecules with specific properties, such as surfactants, adsorbed at the particle surface.<sup>1</sup> It is therefore important for the understanding of colloidal properties based on particle surface conditions that we have the experimental ability to quantitatively detect molecular adsorption in colloidal systems. Many recent investigations have focused on characterizing molecular adsorption for the understanding of the effect of adsorbate–surface interactions on colloidal properties<sup>2–6</sup> as well as the nature of those interactions.<sup>7–9</sup>

It has been recently demonstrated that the second-order nonlinear optical phenomena, such as second harmonic generation (SHG), are dipole allowed for centrosymmetric systems on length scales comparable to or longer than the coherence length, contrary to general thinking.<sup>10a</sup> It is only when the system is centrosymmetric on a length scale much less than the coherence length of SHG, roughly the wavelength of light generated, that SHG is electric dipole forbidden. However, for centrosymmetric particles, in roughly the size range of tens of nanometers to microns, the light induced second-order polarizations from “opposite” sides of the particle do not have opposite phases and therefore do not cancel each other at the interface.

SHG has proven to be a powerful method as seen in its in situ, quantitative characterization of molecular adsorption,<sup>10a–e</sup> including polymer beads,<sup>10b</sup> emulsions,<sup>10b</sup> liposomes,<sup>10d</sup> semiconductor particles,<sup>10e</sup> surfactants,<sup>11a</sup> and biopolymers,<sup>11b</sup> at solid–liquid interfaces. Beyond adsorption, this nonlinear optical approach has been used to measure many static and dynamic interfacial properties.<sup>11c–f</sup>

For molecules that have large second-order polarizabilities that facilitate SHG, such as a dye molecule, the adsorption isotherm can be directly obtained from the second harmonic (SH) intensity generated by the surface adsorbed molecules. SHG can be used to probe the dye molecule surface coverage because of its symmetry characteristics as outlined earlier. When the dye molecules are in the solution phase, they are randomly oriented, and thus, collectively the total second order nonlinear polarization is statistically summed up as zero. However, when the dye molecules are adsorbed onto the surface, they are oriented with respect to the surface normal and thus have nonzero polarization for SHG. Measurement of the SHG signal as a function of the dye concentration in the bulk liquid yields the adsorption isotherm, which allows the determination of the dye molecule adsorption free energy and density.

The adsorption of molecules without a large second order polarizability, which are the great majority of molecules used in colloidal systems, can be detected through competitive adsorption with the dye molecules. The dye molecules with pre-characterized adsorption isotherms are used essentially as an indicator of surface coverage. Experimentally, first, a sufficient concentration of dye is added to the colloid to create saturation coverage on the particle surface and a detectable SHG signal.

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When the target molecule is added to the colloid, it displaces the dye on the surface and its adsorption can be monitored as a decrease of the SHG signal. This approach was first demonstrated by characterizing the adsorption of a methacrylate surfactant,<sup>11a</sup> and later polylysine,<sup>11b</sup> on polystyrene microspheres in water.

In general, this nonlinear optical approach for characterizing molecular adsorption on colloidal surfaces relies on the choice of an appropriate indicator dye molecule. This dye molecule has to give a large SHG signal. It should be soluble in the solvent of the colloid at a specified pH value. Its adsorption free energy should be comparable to or smaller than that of the target molecule so it can be displaced. The dye molecule, malachite green (MG), has been shown to be such a molecule in SHG studies of MG adsorbed to polystyrene sulfate micro-particles in aqueous solution at a pH less than 5.<sup>10a,b</sup> To make this dye molecule a more generally usable indicator of surface coverage, we investigate here its adsorption characteristics on polystyrene particles with different surface compositions, plain polystyrene surface (PPS), polystyrene surface terminated with sulfate groups (PSS), or amine groups (PSA), in water. These particle surfaces are neutral, negatively charged, or positively charged, respectively, under the prescribed pH and aqueous conditions. This investigation also affords the opportunity to examine the effect of different surface charge and composition on molecular adsorption.

The adsorption free energies and densities of the cationic MG on these surfaces with different charge composition can be experimentally measured to assist in our understanding of the nature of the interaction between an adsorbate and the colloidal surface. The nature of the interaction may be chemisorption, hydrogen bonding, hydrophobic, or van der Waals.<sup>12</sup> Numerous experiments have focused on the adsorption of a charged molecule to surfaces of varying charge. Adsorption of an ionic molecule to a surface of similar charge may occur via hydrophobic interactions. Surfactant adsorption to a surface of similar charge has been observed.<sup>2</sup> Cationic and anionic dye molecule adsorption to cationic and anionic activated carbon have been observed.<sup>13</sup> The importance of dispersion interactions in the adsorption of aromatic solutes to activated carbon is noted previously.<sup>9</sup> Cationic dye adsorption to neutral polystyrene polymer<sup>14</sup> as well as neutral polystyrene microspheres<sup>2</sup> and cationic and anionic polystyrene microspheres<sup>15</sup> have been reported. An extensive list of the adsorption of molecules of different charge to a variety of surfaces has been tabulated.<sup>16</sup> The effects of composition and charge have been investigated using SHG. It was shown that the fraction of charged phospholipids in the composition of a liposome bilayer has a very large effect on the adsorption of MG to the liposome–aqueous interface.<sup>10d</sup>

As evidenced by the adsorption of molecules on a surface of similar charge, the electrostatic interaction of the surface and adsorbate are not the only interactions that must be considered. Not only are other attractive forces between the surface and adsorbate of importance but also their interaction with the solvent is important as well. The solvent affects adsorption in three ways: (1) how it interacts with the solute or molecule (solubility), (2) how it interacts with the surface, and (3) how it interacts with the adsorbed molecules.<sup>12</sup> Molecules that have limited solubility in water have hydrophobic character and thus will be attracted to hydrophobic surfaces or hydrophobic parts of surfaces.<sup>3</sup> The overall attraction to the surface is a balance of the forces between the solvent, surface, and adsorbates as well as any interadsorbate interactions.

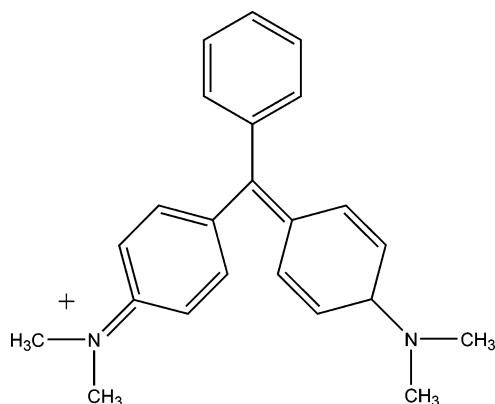
In this report, we will show that MG, a cationic dye, adsorbs to all three surfaces regardless of surface charge. The nonlinear optical measurements allow the determination of the adsorption free energies and maximum adsorption densities. Furthermore, we will show that the relative magnitude of the SH intensities from MG adsorbed on the three surfaces can be used, in combination with a model calculation, to determine the relative tilt angles of the adsorbate on the three surfaces. The quantitative information on adsorption free energies, densities, and configurations reveals the nature of the adsorbate–substrate interactions at the liquid–solid interface in the aqueous colloids.

## 2. Experimental Section

**2.1. Second Harmonic Generation.** The details of the experimental set up for the SHG measurement of adsorption in colloidal systems have been reported previously.<sup>11</sup> Briefly, the source for the fundamental light of SHG is a Ti:sapphire laser (Coherent Mira Seed, oscillator only) pumped by an Ar ion laser (Coherent Innova 310) which provides nominally 50 fs long pulses at 840 nm with less than 4 nJ pulse energy at a 76 MHz repetition rate. These nonamplified femtosecond laser pulses are used because they provide the high peak power necessary for the nonlinear optical process, while having a low pulse energy that minimizes heating and photochemical effects from light absorption by the sample. The fundamental-light beam is focused by a 2" focal-length lens into a liquid jet sample. At the focus the peak intensity of the light is approximately  $6 \times 10^9$  W/cm<sup>2</sup>. The laser beam, after exiting the sample, consists of the fundamental and the Second Harmonic in the same direction. The lens after the sample collects all light from the sample at  $\pm 27^\circ$  with respect to the Z axis (defined by the propagation of the fundamental). It then passes through a series of colored filters (for filtering out the fundamental as much as possible) and enters a monochromator where the second harmonic wavelength is allowed to pass through. The rectangular nature of the monochromator does not allow all light collected to enter it. In the Y–Z plane (perpendicular to the laboratory table),  $\pm 27^\circ$  enters, but in the X–Z plane (parallel to the table) only  $\pm 3^\circ$  enters the monochromator. The SH signal is then detected by a photomultiplier (Hamamatsu R585). It is then amplified and processed through a correlated photon counting system that suppresses statistical random noise with a discrimination voltage of  $-100$  mV when the PMT is powered at 1300 V. The signal collected is an average over 1 s.

For polarization specific SHG experiments, a thin polarizer (Sheet Polarizer, Melles Griot) was placed before the sample and another polarizer (Karl Lambrecht, Glan Taylor Prism Polarizer, extinction ratio  $< 0.0005$ ) mounted on a rotating stage was placed before the detection system. The output of the Ti:sapphire laser is linearly polarized in the *p* direction, parallel to the X axis of the optical table defined as the X–Z plane in the laboratory frame (the light beam direction is defined as the Z axis). A reading of  $0^\circ$  on the polarizer mount corresponds to *p*-polarized SH light.

The colloidal solution is formed into a continuous liquid jet through which the laser beam intersects. A reservoir of approximately 250 mL of the sample is prepared and stirred continuously with a magnetic stirrer. A small volume of the sample is then pumped out of the reservoir through Nalgene tubing and forced through a pressed stainless steel nozzle (1/16 in. inner diameter) to form a liquid jet. A pumping speed of 2.5 mL/s is used to ensure that the entire volume of the sample quickly circulates through the setup. Using the liquid jet avoids any window effects from a sample holder, including SH generated by the windows, or sample burning onto the windows.



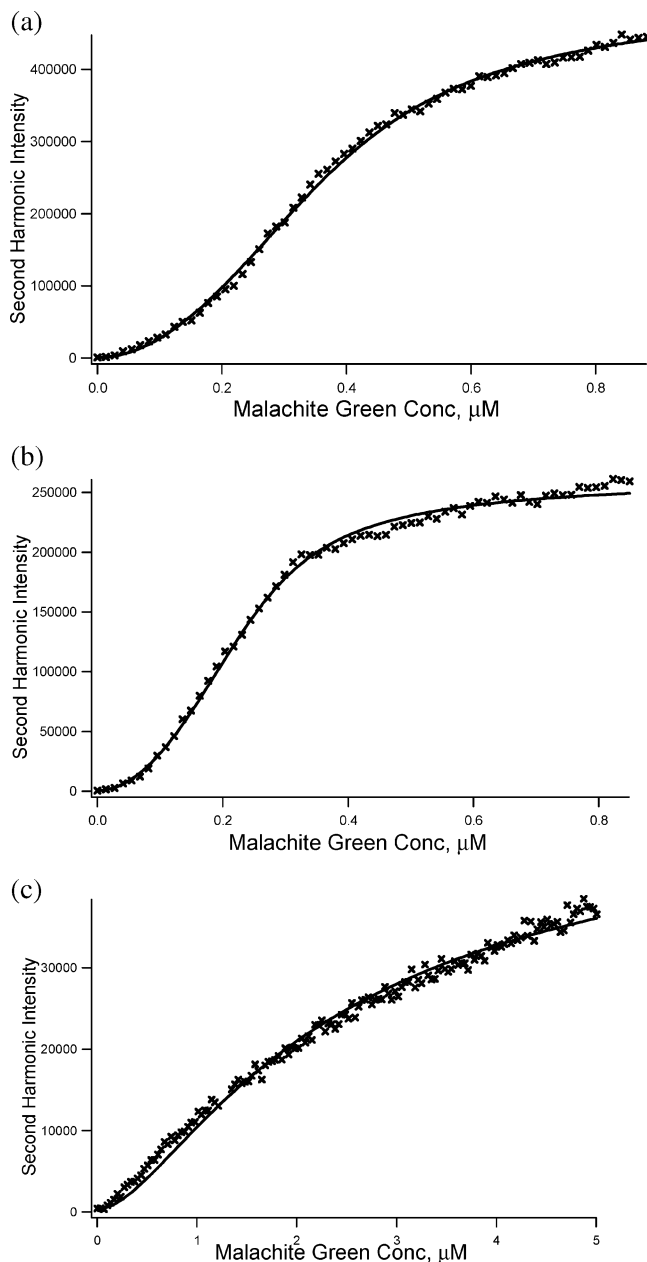
**Figure 1.** Malachite green cation structure. The equivalent resonance structures with the positive charge on either one of the N atoms result in a  $C_{2v}$  symmetry. The Cartesian coordinates in the molecular frame are set to have the  $z'$  axis parallel to the  $C_2$  rotation axis and the  $y'$  axis out of the molecular plane.

A digital titration buret (CAT Contiburet) containing the dye solution is used to add the dye molecules to the colloid reservoir. This allows the dye addition to be synchronized with the data collection system and enables the collection of SH intensity as a function of dye concentration added. In the titration experiment, a 4-s interval is allowed between the dye aliquots to ensure the equilibrium state of the sample can be reached. The total titration volume is kept below 0.01% of the reservoir volume to avoid dilution effects. The recording of an adsorption isotherm in the form of SH intensity as a function of the dye concentration typically is completed in less than 3 min.

The colloidal solutions are made of 0.01% polystyrene microspheres in deionized water. HCl is added to adjust their pH to 4.5. Three types of microspheres (Polysciences, Inc.) are used: (1) "plain" polystyrene microspheres (PPS) (diameter  $1.05 \pm 0.03 \mu\text{m}$ ) which carry an unspecified but negligible amount of negative charges due to the preparation procedure of the microspheres, but are considered plain in terms of its surface characteristics; (2) polystyrene sulfate microspheres (PSS) (diameter  $1.09 \pm 0.03 \mu\text{m}$ ) which are negatively charged due to an enhanced sulfate ( $-\text{SO}_4^-$ ) concentration on the surface; and (3) polystyrene amino microspheres (PSA) (diameter  $0.91 \pm 0.02 \mu\text{m}$ ) which contain amine groups ( $-\text{NH}_2$ ) on the surface, these groups are positively charged at  $\text{pH} < 5$ . The different diameters were taken into account in the sample preparation so that the total surface areas for all samples are kept constant at  $6.0 \times 10^8 \mu\text{m}^2/\text{mL}$ .

MG, structure shown in Figure 1, was purchased from Sigma-Aldrich and used without further purification. MG exists in a cationic form at pH 4.5. At high pH, MG has indicator properties and is converted into a neutral, colorless form. For this reason, the experiments reported here are performed at pH 4.5.

**2.2. Centrifugation-Separation Method.** For calibration purposes, the amount of MG molecules adsorbed on the particles may also be made independently using a centrifugation-separation method. For these experiments, two samples are prepared. One contains only dye. The other, the colloid, contains the same dye concentration and 0.01% microparticles. Dye concentrations used are high enough to cause saturation coverage over the surface of PPS and PSS ( $1 \mu\text{M}$  MG) and PSA ( $4.90 \mu\text{M}$ ). Optical absorbance at 615 nm by the dye-only solution is first measured, using a spectrophotometer (Beckmann Coulter DU640) with a resolution of  $\pm 2$  nm, to establish the dye concentration—absorption calibration. The absorbance at 615 nm of the supernatant of the colloidal samples following



**Figure 2.** Second harmonic intensity detected as a function of the added concentration of malachite green dye in the colloid solutions of (a) plain polystyrene microspheres (PPS); (b) polystyrene sulfate microspheres (PSS); and (c) polystyrene amino microspheres (PSA). The SH intensity is expressed in number of photon counts per second.

centrifugation (Sorvall Biofuge pico microcentrifuge, 12 000 rpm, 4 min) is then measured. A comparison of this absorbance with the absorbance from the dye-only solution reveals how much dye is depleted from the solution by adsorption onto the microspheres and allows the calculation of the number of dye molecules adsorbed per microsphere.

### 3. Results and Analysis

**3.1. Second Harmonic Generation.** Second harmonic intensity detected as a function of the MG dye concentration in the colloidal solution for the three colloidal systems is shown in Figure 2. The SH intensity is expressed as the number of photon counts per second. In all three cases, as the quantity of dye added into the colloidal solution increases, the SH intensity increases. For the PSS colloid, Figure 2b, the SH intensity rises rapidly and begins to level off when the MG concentration



**TABLE 1: Maximum Density, Free Energy of Adsorption, and Relative 2nd Order Polarizability for MG on PPS, PSS, and PSA Microspheres Measured Using SHG**

surface	density (MG/ $\mu\text{m}^2$ ) <sup>a</sup>	$K$ (adsorption equilibrium constant)	$\Delta G$ (kcal/mol)	polarizability per MG <sup>b</sup>
PPS	$3.71 \pm 0.06 \times 10^5$	$1.75 \pm 0.13 \times 10^9$	$-12.39 \pm 0.15$	0.90
PSS	$2.54 \pm 0.05 \times 10^5$	$2.44 \pm 0.24 \times 10^9$	$-12.67 \pm 0.16$	1.00
PSA	$2.63 \pm 0.87 \times 10^5$	$5.62 \pm 0.19 \times 10^7$	$-10.46 \pm 0.08$	0.37

<sup>a</sup> The number of MG per sphere for PSA was obtained using the centrifugation method at  $4.90 \mu\text{M}$  MG. This number is lower than but close to the maximum density. <sup>b</sup> Relative value per adsorbed MG molecule.

passes  $\sim 0.3 \mu\text{M}$ , whereas in other SHG work, the saturation was observed to occur at higher concentration,<sup>10b</sup> perhaps due to different surface characteristics of the particles. As the description below shows, the SH intensity is proportional to the square of the dye surface coverage, the plateau in the SH intensity corresponds to saturation of the dye surface coverage. The SH response from the solution of PPS titrated with MG shown in Figure 2a also displays an increase with the increase of the dye concentration, though less rapidly with a later saturation than PSS. The SH intensity also increases when the PSA colloid is titrated with MG, Figure 2c, but with a much slower rate than the other two colloids. For the PSA particles, the point of surface adsorption saturation lies well above  $5 \mu\text{M}$  MG.

The intensity of the second harmonic,  $I$ , is related to the coverage of MG,  $\theta_D$ , on the microsphere surface through

$$I = B + A(a + b\theta_D e^{i\phi})^2 \quad (1)$$

In eq 1, term  $B$  is used to account for any background signal. The contribution to SHG from the solid–liquid interface, term  $A$ , arises from two sources: dye molecules adsorbed on the surface and the particle itself. The latter is represented by the coefficient  $a$ . The adsorbed dye contribution is represented by the coefficient  $b$  and is proportional to  $\theta_D$ .  $e^{i\phi}$  depicts the relative phase between the  $a$  and  $b$  terms.

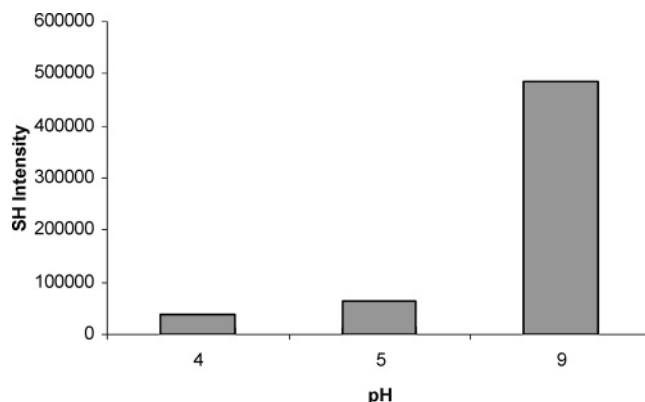
The expression for the surface coverage of the adsorbed dye can be derived from a modified Langmuir adsorption model based on the dye adsorption–desorption equilibrium with the equilibrium constant  $K_D$  and including the bulk depletion of MG<sup>10b</sup>



$$\theta_D = [(C_D + N_{\text{max}}^D + 55.5/K_D) - [(C_D + N_{\text{max}}^D + 55.5/K_D)^2 - 4C_D N_{\text{max}}^D]^{1/2}] / 2N_{\text{max}}^D \quad (3)$$

Here  $C_D$  is the added concentration of MG to the sample and  $N_{\text{max}}^D$  is the maximum number density of dye molecules that may adsorb onto the microsphere surface.  $N_{\text{max}}^D$  and  $K_D$  may be obtained by a nonlinear least-squares fit of the experimentally observed SH intensity as a function of  $C_D$  to eqs 1 and 3.  $K_D$  can then be used to determine the adsorption free energy of the dye molecule on the surface using  $\Delta G = -RT \ln K$ .

The nonlinear least-squares fits of  $I(C_D)$  for all three colloidal systems have been done and are shown as the solid lines in Figure 2. For all three colloidal systems, the bulk contribution of the microspheres is negligible, and  $a$  and  $\phi$  are essentially zero. The maximum MG adsorption density and the adsorption free energy at the ambient temperature  $22^\circ\text{C}$  on each of the three microspheres are listed in Table 1. For the amine-terminated surface data, the nonlinear least-squares fit did not allow an uncorrelated determination of the two parameters. Subsequently, an independent measurement of  $N^D$  at  $4.90 \mu\text{M}$

**Figure 3.** Comparison of second harmonic intensity of malachite green adsorbed to polystyrene amino microspheres at different pH values.

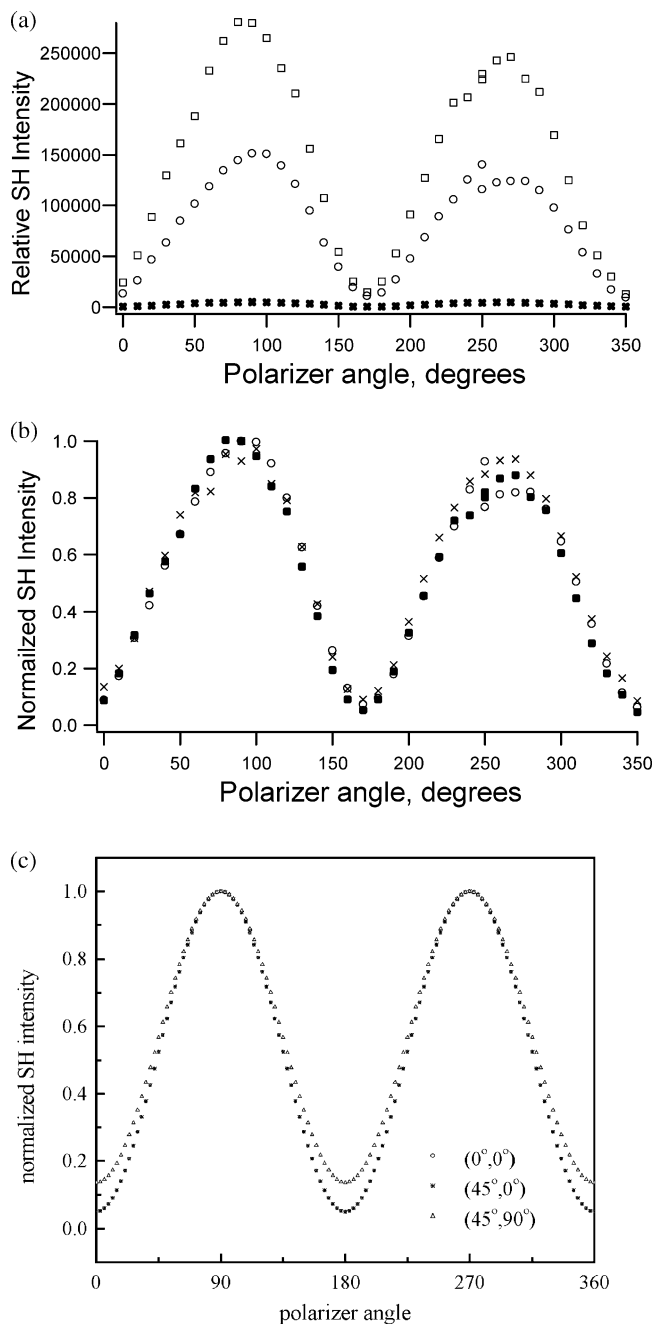
MG was made using the centrifugation-separation method, and it was found to be  $2.63 \times 10^5 \text{ MG}/\mu\text{m}^2$ . Using the adsorption density determined for the PSA colloid from the centrifugation method in the SH intensity fitting, the equilibrium constant was determined that resulted in a  $-10.46 \pm 0.08 \text{ kcal/mol}$  free energy of adsorption. An earlier study<sup>10b</sup> yielded the value of  $5 \times 10^5$  for the maximum number of  $\text{MG}/\mu\text{m}^2$  adsorbed, which is a factor of 2 larger than the value obtained in this study, perhaps due to different surface characteristics of the particles.

Table 1 also includes the relative magnitude of the second-order polarizability of adsorbed MG molecules at  $840 \text{ nm}$ , denoted as  $\beta$ , on the three surfaces. The SH intensity, when the total particle surface area is set as constant, is related to the adsorbed MG  $\beta$  and  $N^D$  as

$$I \propto N^2 \beta^2 \quad (4)$$

Using the maximum intensities observed in Figure 2 and MG densities reported in Table 1, the relative magnitude of  $\beta$  of the three different types of adsorbed MG on the three different surfaces can be estimated accordingly. The values listed in Table 1 have been normalized by setting the highest value, that of MG on PSS, as unity.

For the amine-terminated surface, the effect of pH on MG adsorption is also of interest. Below pH 5, the amine groups are in  $-\text{NH}_3^+$  form and this surface is positively charged. As the pH is raised, it becomes neutral ( $-\text{NH}_2$ ). An experiment was done in which the MG concentration was held constant while the pH of the colloidal solution was increased (by adding aqueous  $0.01 \text{ N NaOH}$ ) from the initial value of pH 4.5. Figure 3 shows the SH intensity level as a function of pH. As the pH was increased, the intensity increased. Within seconds at  $\text{pH} > 5$ , MG begins to convert into a colorless, neutral form.<sup>17</sup> When this occurs, MG loses its ability to produce resonantly enhanced SHG at the wavelength used here causing a decrease in the SH intensity. For this reason, an adsorption isotherm of the amine surface under neutral surface charge was not collected. The results presented in Figure 3 are results observed upon the pH increase before the MG conversion occurs.



**Figure 4.** SHG intensity polarization dependence with p-polarized fundamental: (a) the SH intensity detected as a function of the second harmonic light polarization for the three surfaces, PPS =  $\square$  or  $\blacksquare$ , PSA =  $\times$ , and PSS =  $\circ$ ; (b) the data in (a) plotted with the maximum intensities normalized at the same value; (c) SH intensity (normalized) as a function of its polarization calculated using the model for different orientation angles ( $\theta, \psi$ ) of  $(0^\circ, 0^\circ)$ ,  $(45^\circ, 0^\circ)$ , and  $(45^\circ, 90^\circ)$ .

The polarization dependence of the SHG was investigated. Figure 4a shows the relative SH intensity detected while changing the output SH light polarizer angle for the three surfaces each with a saturated MG coverage ( $1.0 \mu\text{M}$  for PPS and PSS, and  $4.9 \mu\text{M}$  MG for PSA). The fundamental light is p-polarized (parallel to the optical table, and corresponding to the  $0^\circ$  and  $180^\circ$  in the figure). In Figure 4b, the SH intensities are normalized so that the highest points of all three curves are set as unity. Interestingly, all three surfaces show the same maxima and minima, with nearly identical ratios! The maxima at  $90^\circ$  and  $270^\circ$  correspond to s-polarized SHG reaching the detector. This shows that in the experimental configuration the

**TABLE 2: Maximum Density for MG on PPS, PSS, and PSA Microspheres Measured Using Centrifuge-Separation Method<sup>a</sup>**

surface	absorbance of MG only	abs. of MG after microspheres removal	density (MG/ $\mu\text{m}^2$ )
PPS	0.178	0.138	$2.14 \pm 0.29 \times 10^5$
PSS	0.178	0.143	$1.85 \pm 0.23 \times 10^5$
PSA	0.857	0.809	$2.63 \pm 0.88 \times 10^5$

<sup>a</sup> Absorbance values were measured at 615 nm.

SHG experiment is conducted, the maximum SH output (s-polarized) is polarized perpendicularly to the incoming fundamental light (p-polarized). This polarization dependence is important in the discussion of the adsorption configuration (section 4.3).

**3.2. Centrifugation-Separation Method.** The centrifuge-separation method provides an independent measurement of the amount of MG adsorbed to the three surfaces. Absorbance values at 615 nm for solutions containing only MG were recorded and are presented in Table 2. Samples were also prepared at the same MG concentrations with microspheres. After the microspheres were removed via centrifugation, the absorbance at 615 nm of the supernatant was measured. From the difference between the two absorbance measurements, the MG density on each surface was found to be  $2.14 \times 10^5$  MG/ $\mu\text{m}^2$  for PPS,  $1.85 \times 10^5$  MG/ $\mu\text{m}^2$  for PSS and  $2.63 \times 10^5$  MG/ $\mu\text{m}^2$  for PSA.

## 4. Discussion

**4.1. Adsorption Free Energy.** A cationic molecule may interact with a surface through electrostatic and/or van der Waals forces depending on the surface composition. For a negatively charged surface, the attraction to the surface should be strong, resulting in a low (or large negative) free energy of adsorption. For a neutral surface, adsorption occurs based on van der Waals forces. This attraction to the surface should be weaker than a charge-charge interaction. For cationic surfaces, due to the like charges of the surface and molecule, a repulsive force exists. The cationic molecule may still be attracted to the polymeric part of the surface through van der Waals forces, but the repulsive charge-charge interaction may reduce the adsorbate-substrate binding and change the conformation of adsorption. Adsorption of a cationic molecule will result from a balance of repulsion and attraction between the surface and molecule. According to this model of adsorption, it is expected that the free energy of adsorption should have the largest absolute magnitude for the interaction with the anionic surface and the smallest for the cationic surface. This is indeed the case. The  $\Delta G$  for the adsorption of the MG cation is  $-12.67$  kcal/mol for the anionic, sulfate-terminated surface, which compares to the value of  $-11.1$  kcal/mol found in previous studies,<sup>10b</sup>  $-12.39$  kcal/mol for the plain and  $-10.46$  kcal/mol for the amine-terminated cationic surface.

The difference in magnitude between electrostatic and van der Waals interactions should be large, yet for the sulfate-terminated and plain surfaces, only a small difference in  $\Delta G$  is observed. As pointed out by Somasundaran et al.,<sup>3</sup> the free energy of adsorption has many contributions including terms originating from charge-charge interactions, covalent bonding, adsorbate-adsorbate hydrophobic (van der Waals) interactions, hydrophobic adsorbate-substrate interactions, hydrogen bonding, and solvation. Others have considered the balance of

electrostatic as well as van der Waals forces in adsorption of molecules.<sup>7–9</sup> The importance of these contributions depends on the properties of specific adsorbates and surfaces as well as the solvent used. For the MG adsorption to the negative surface, the contribution of the attractive electrostatic term will be large in comparison with the weaker van der Waals adsorbate–substrate interaction on the neutral surface. However, contributions from other terms such as the solvation term offset the difference and results in similar  $\Delta G$ 's. The negative charges on the sulfate-terminated surface are much more strongly solvated by the water solvent molecules than the plain, neutral surface. The loss of the contribution from the solvation term must have also contributed to the reduction of the adsorption free energy on the amine-terminated, positively charged surface.

**4.2. Adsorption Density.** For the sulfate-terminated surface, as the adsorption is dominated by opposite charge attractive interaction, it is reasonable to expect that, as each negative surface charge would host one cation, the adsorbate density should be approximately equal to the negative charge density on the surface. The surface charge density of PSS can be estimated using the Gouy–Chapman model analysis<sup>18</sup> of the zeta potential which was measured at pH 2. The model generated a value of  $5.8 \times 10^4$  sulfate groups per  $\mu\text{m}^2$ . This value was derived from the calculated surface potential at the sheer surface, not the actual microsphere surface, and serves as the lower boundary of the true surface density.<sup>10b</sup> The maximum MG density on the PSS surface measured from the SHG data is  $2.54 (\pm 0.05) \times 10^5$  MG/ $\mu\text{m}^2$ . This number is close to but slightly higher than the less accurate  $1.85 (\pm 0.23) \times 10^5$  MG/ $\mu\text{m}^2$  measured by using the centrifuge-separation technique. Both numbers are larger than the estimated lower boundary of the sulfate group density and are consistent with the model that each negative charge site has a cationic MG adsorbed. The value of the electrostatic potential at the surface of the PSS microsphere has been determined using SHG.<sup>10b</sup> Furthermore, the value of the surface potential and the potential at the shear plane obtained from electrophoresis measurements have been quantitatively related using a theoretical model in which the viscosity changes continuously rather than discontinuously in the shear zone.<sup>10b,19</sup>

At the measured maximum density of adsorbed dye, each MG molecule on PSS takes up a surface area of about  $393 \text{ \AA}^2$ . This area per MG molecule is somewhat larger than the  $200 \text{ \AA}^2$  disk shape of MG defined by the van der Waals radius of  $8 \text{ \AA}$ . This observation that the MG molecule occupies an area much larger than its size is also consistent with the model that on this surface, opposite charge attraction interaction is responsible for adsorption so the adsorbate density is set by the surface charge density. Also at the measured maximal surface density, two cations are separated at a distance of  $19 \text{ \AA}$  on the surface. At this distance, without including the charge screening by the polar solvent molecules and ions, the repulsion between the two cations is only  $0.22 \text{ kcal/mol}$ . It is likely that the point-to-point charge attraction between a MG adsorbate and a surface sulfate group allows more motion of the MG on the surface. A MG molecule, with a diameter of about  $13 \text{ \AA}$ , under complete free rotation motion around the sulfate charge, however, would require an area more than  $500 \text{ \AA}^2$ , somewhat larger than the measured  $393 \text{ \AA}^2$ .

On the plain surface, the maximum cationic MG density ( $3.71 (\pm 0.05) \times 10^5$  MG/ $\mu\text{m}^2$ ) is higher than that on the negatively charged surface. Again, using the centrifuge technique, a slightly lower value of  $2.14 (\pm 0.29) \times 10^5$  MG/ $\mu\text{m}^2$  was found. In both types of measurements, the fact that the density on the plain

PPS surface is higher than the negatively charged PSS surface is consistent. The limiting factor for adsorption to this surface is the amount of surface area available since the attractive force promoting adsorption is through van der Waals interactions between the dye and the surface. The maximum adsorption density corresponds to  $270 \text{ \AA}^2$  per adsorbed MG. This value is also larger than the disk size of MG, allowing any adsorption configuration that can maximize MG's interaction with the polystyrene substrate.

The density measured for the positively charged amine surface using the centrifuge technique at  $4.90 \mu\text{M}$  solute concentration is already  $2.63 \times 10^5$  MG/ $\mu\text{m}^2$ . This number may be lower than, but should be close to, the saturation coverage density. The saturation coverages measured by both SHG and centrifugation methods are close to each other and comparable to the MG adsorption densities on the other two types of surfaces. The area that each dye takes up,  $380 \text{ \AA}^2$ , is larger than the physical size of MG. This area allows any configuration that is required to maximize the adsorption interaction.

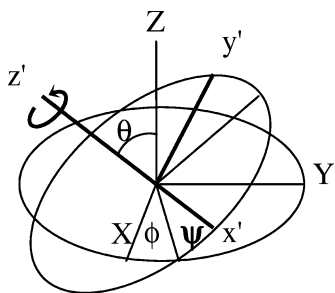
According to the zeta potential measurements, it is found that there are  $9.6 \times 10^4$  amine groups per sphere. Adding this number of amine positive charges to the number of MG positive charges on the surface, the overall number of positive charges on the surface is estimated at  $3.59 \times 10^5$  per sphere, resulting in a minimum intercharge distance of  $19.4 \text{ \AA}$  if the charges are distributed evenly on the surface. At this distance, the pair of two positive charges without screening would have a repulsion potential of  $0.22 \text{ kcal/mol}$ , a number that can be overcome by van der Waals interactions.

**4.3. Adsorption Geometry.** In all three cases, the SH intensity generated is completely from the adsorbed MG molecules on the surfaces. Since the adsorption occurs on the surface of a particle with spherical symmetry, SHG depends in general only on the orientation of the adsorbed molecules. The adsorption configuration of the dye molecule, defined by the tilt angle from the local surface norm and the rotation angle of the molecule around the principal rotation axis, affects the intensity, the scattered angle, and the polarization angle of the SH light. A detailed account of the relationship between the adsorption geometry and the SH light characteristics will be published elsewhere.<sup>20</sup> Here we report the limited but useful information that can be obtained on the adsorption geometry from examining the SH intensity, in relative magnitude, from the three different surfaces. From the discussion on the adsorption free energies and densities, one can perceive that different adsorption configurations exist for the three surfaces.

Table 1 lists the relative 2nd order polarizability per adsorbed MG molecule on the three surfaces. A MG molecule adsorbed to the plain surface exhibits only 90% of the polarizability as one on the sulfate surface, whereas MG on the amine surface gives much less, only 37%. These relative values can be understood from the adsorption geometry.

The second-order susceptibility  $\chi_{ijk}$  is the ensemble average of the contributing individual molecular polarizability elements  $\beta_{ijk}$ .<sup>21</sup> For molecules such as MG with  $C_{2v}$  symmetry, the nonzero  $\beta$  tensor elements, in the  $x'y'z'$  molecular frame, are  $\beta_{zzz}$ ,  $\beta_{zzx}$ ,  $\beta_{zzx} = \beta_{xxz}$ ,  $\beta_{zyy}$ , and  $\beta_{zyy} = \beta_{yzy}$ . The  $420 \text{ nm}$  SH light is in resonance with a transition along the MG molecular  $z'$  axis (the  $C_2$  rotation axis),<sup>22</sup> thus reducing  $\beta$  to three independent elements  $\beta_{zzz}$ ,  $\beta_{zzx}$ , and  $\beta_{zyy}$ . A previous work<sup>23</sup> has measured the SHG from MG adsorbed on silica and found that  $\beta_{zzx}$  was the dominant contributor. The following analysis is based on the assumption that MG SHG can be represented by the dominant element  $\beta_{zzx}$ .





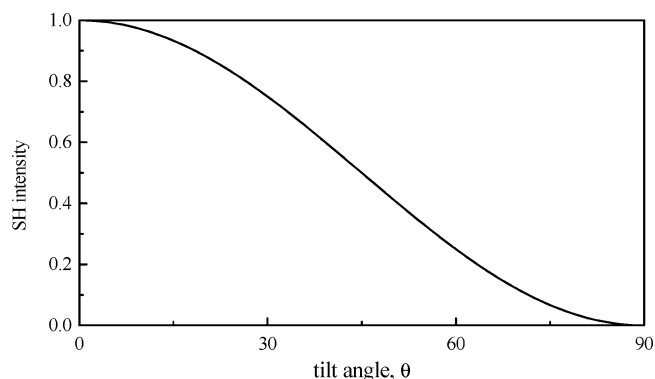
**Figure 5.** MG adsorption configuration defined by  $\phi, \theta, \psi$ .

In general, MG orientation on a particle surface can be described by three angles (Figure 5).  $\phi$  is the azimuthal rotation angle on the local  $X$ – $Y$  plane about the local  $Z$  axis. The angle  $\phi$  gets averaged out on an isotropic plane such as the surface of the spherical particle.  $\theta$  is the tilt angle between the molecular  $z'$  axis and the local surface normal  $Z$  axis.  $\psi$  represents the molecular rotation about the molecular  $z'$  axis.

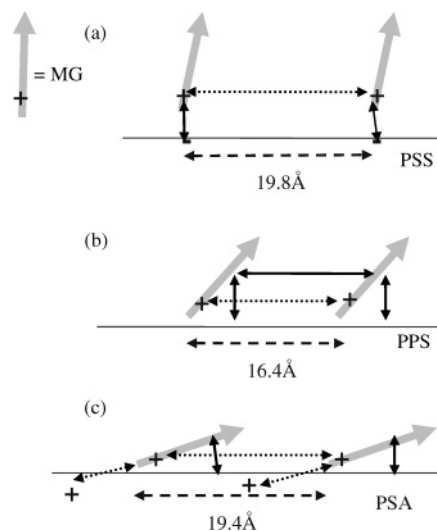
The effect of the adsorption geometry defined by the set of angles  $(\theta, \psi, \phi)$ , or by  $(\theta, \psi)$  since  $\phi$  is averaged out on the isotropic surface, on the SHG polarization dependence can be theoretically modeled.<sup>20</sup> A calculation has been performed to simulate the polarization angle dependence of the SH light generated by p-polarized fundamental within scattering angles of  $\pm 27^\circ$  along the  $Y$ – $Z$  plane and  $\pm 3^\circ$  along the  $X$ – $Z$  plane which mimics the experimental collection of the SH light by the monochromator with an inlet slit (see section 2). The SH intensity at the different polarization angles calculated for the above experimental configuration is displayed in Figure 4c for three different combinations of  $(\theta, \psi)$ . It appears that the polarization dependent intensity, in relative magnitude, of the SH light collected for this particular experimental configuration is rather insensitive to the molecular orientation angles. All three sets of orientation angles give similar polarization dependences. This calculation on one hand explains the nearly identical SH polarization patterns observed for the three different surfaces—even though the orientation angles of the adsorbed MG could be very different on the three surfaces, they would still give the same polarization angle pattern. On the other hand, it also shows that the SH polarization pattern measured under this particular experimental configuration does not carry much information with regard to the adsorption geometry.

The information that can be used to decipher the adsorption geometry lies with the relative magnitude of the SH intensities. The same model<sup>20</sup> shows that the s-polarized SH light (the peak of the polarization angle pattern) intensity depends directly on the tilt angle  $\theta$ . Figure 6 shows how the SH intensity detected under the same experimental configuration may vary according to the angle  $\theta$ . Qualitatively, the larger the tilt angle  $\theta$  is, the smaller the SH intensity will be.

Based on the calculated SH intensity variation in Figure 6 and the experimentally observed difference in SH intensity,  $\theta$  may be estimated for the three surfaces relative to one another.  $\beta$  was calculated from the different experimental SH intensities. Since  $\beta^2$  is proportional to the intensity, the relative values of  $\beta^2$  derived from Table 1 will be used together with Figure 6 to obtain the change of  $\theta$  from one surface to another. To determine the absolute values of the three  $\theta$  angles, an assumption is made as to the  $\theta$  for MG on PSS. MG may not adsorb at exactly  $\theta = 0^\circ$ . Previously, for the silica–air interface, MG was found to adsorb with a small tilt angle of  $8^\circ$ .<sup>23</sup> Thus, we assume a  $10^\circ$  angle for  $\theta$  for MG adsorption on PSS. Based on the relative values of  $\beta^2$ ,  $\theta$  was found to be  $10^\circ$ ,  $28^\circ$ , and  $69^\circ$  for MG on PSS, PPS and PSA, respectively.



**Figure 6.** Model calculation of the SH intensity, in relative magnitude, as a function of the MG tilt angle on the surface. The calculation is for the solid angles specified by the experimental configuration described in the text and the s-polarized SH light generated with p-polarized fundamental.



**Figure 7.** Proposed schematic representation of adsorption density and configuration for MG on (a) PSS, (b) PPS, and (c) PSA. Dotted arrows represent repulsive interactions, solid arrows represent attractive interactions.

**4.4. Adsorption of the Cationic MG on the Three Differently Charged Surfaces and the Effect of the pH.** Figure 7 summarizes the different adsorption densities and configurations for MG on the three surfaces. For the sulfate surface, shown in Figure 7a, the most reasonable configuration would have the positive charge localized at one of the nitrogen atoms interacting with the negatively charged surface groups. The configuration would be standing relatively upright ( $\theta \sim 10^\circ$ ) with respect to the surface normal to maximize the electrostatic interaction.

On the plain surface (Figure 7b), the dominating interaction is the van der Waals interaction. For this surface, the dye molecule will be at an angle ( $\theta \sim 28^\circ$ ) in an attempt to maximize its hydrophobic interaction with the surface while keeping the positive charge in a water solvated environment. It also may have the benefit of attractive interadsorbate interactions.

The dye on the cationic surface has a similar density of MG to other surfaces but gives a much lower SH intensity due to the much more tilted configuration ( $\theta \sim 69^\circ$ ) (Figure 7c). A flatter configuration maximizes the hydrophobic interactions with the polystyrene surface. The charges already present on the surface cause a lower adsorption density but allow a larger area per dye than on the plain surface. The lower density reduces like-charge repulsion, and the larger area enables the molecule to tilt more so that the phenyl groups may maximize its

interaction with the aromatic rings of the polystyrene surface while keeping the MG charge solvated.

One of the determining factors for adsorption to the cationic surface is the like-charge repulsion on the surface. The amine groups on the surface are either positively charged ( $-\text{NH}_3^+$ ) or neutral ( $-\text{NH}_2$ ) with the relative concentrations of the two groups determined by the pH of the solvent. At high pH, the amine-terminated surface becomes neutral from the positively charged surface at low pH, as a result of change from  $-\text{NH}_3^+$  to  $-\text{NH}_2$ . A corresponding change of adsorption configuration and density should be observed through the observation of a change in the SH intensity. The increase in intensity as a function of pH seen in Figure 3 is consistent with the anticipated changes of adsorption of the dye molecules. Indeed, the SH intensity greatly increased from pH = 4 to 9 to the level similar to that observed for the plain surface.

## 5. Summary

It is shown that optical second harmonic generation can be used to characterize the adsorption of a cationic dye molecule at the aqueous interface of not only negatively charged colloids but neutral and positively charged colloids as well. Adsorption of the cationic dye MG occurs on all three polystyrene microspheres surfaces, plain, negatively charged (sulfate-terminated), and positively charged (amine-terminated). The nonlinear optical approach allows the measurement of the adsorption free energy, the adsorption density, and even the adsorption configuration. Independent measurements of the adsorption density for the dye on all three surfaces were made using a centrifuge-separation technique. These results support the SHG measurements.

The observed adsorption characteristics can be rationalized by the different dye-surface interactions on the three different surfaces. For adsorption onto an oppositely charged surface, electrostatic interactions dominate and the dye adsorbed with a large negative  $\Delta G$  with a density determined by the surface negative charges. The dye most likely is adsorbed in a configuration, upright, allowing maximum rotational degrees of freedom. Adsorption to a neutral surface, originating from van der Waals interactions, appears to have a  $\Delta G$  only slightly more positive. The adsorption density, however, is higher, and the molecule is more tilted for maximizing the adsorbate-surface and possibly interadsorbate interactions. For a similarly charged surface, adsorption still occurred, but with a much flatter configuration. For the cationic surface, the SH intensity was found to increase as the surface charge density decreased indicating a change in adsorption configuration.

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## References and Notes

- (1) Somasundaran, P.; Markovic, B.; Yu, X.; Krishnakumar, S. In *Handbook of Surface and Colloid Chemistry*; Birdi, K. S., Ed.; CRC Press LLC: Boca Raton, FL, 2003; p 387.
- (2) Zollers, R. L. In *Surfactant Science Series*; Schwarz, J. A., Contescu, C. I., Eds.; Marcel Dekker: New York, 1999; Vol. 78, p 645.
- (3) Somasundaran, P.; Shrotri, S.; Huang, L. *Pure Appl. Chem.* **1998**, *70*, 621.
- (4) Aray, Y.; Marquez, M.; Rodriguez, J.; Coll, S.; Simon-Manso, Y.; Gonzalez, C.; Weitz, D. A. *J. Phys. Chem. B* **2003**, *107*, 8946.
- (5) Matijevic, E. *Stud. Surf. Sci. Catal.* **1999**, *120A*, 847.
- (6) Gonzalez-Garcia, C. M.; Gonzalez-Martin, M. L.; Gallardo-Moreno, A. M.; Gomez-Serrano, V.; Labajos-Broncano, L.; Bruque, J. M. *J. Colloid Interface Sci.* **2002**, *248*, 13.
- (7) Radovic, L. R.; Ume, J. I.; Scaroni, A. W. In *Fundamentals of Adsorption*; Le Van, M. D., Ed.; Kluwer Academic: Norwell, MA, 1996.
- (8) Muller, G.; Radke, C. J.; Prausnitz, J. J. *Colloid Interface Sci.* **1985**, *103*, 466.
- (9) Radovic, L. R. In *Surfaces of Nanoparticles and Porous Materials*; Schwarz, J. A., Contescu, C. I., Eds.; Marcel Dekker: New York, 1999; Vol. 78.
- (10) (a) Wang, H. F.; Yan, E. C.; Borguet, E.; Eienthal, K. B. *Chem. Phys. Lett.* **1996**, *259*, 15. (b) Wang, H.; Yan, E. C. Y.; Liu, Y.; Eienthal, K. B. *J. of Phys. Chem. B* **1998**, *102*, 4446. (c) Wang, H.; Borguet, E.; Yan, E. C. Y.; Zhang, D.; Gutow, J.; Eienthal, K. B. *Langmuir* **1998**, *14*, 1472. (d) Liu, Y.; Yan, E. Y. C.; Eienthal, K. B. *Biophys. J.* **2001**, *80*, 1004. (e) Liu, Y.; Dadap, J. I.; Zindars, D.; Eienthal, K. B. *J. Phys. Chem. B* **1999**, *103*, 2480.
- (11) (a) Wang, H.; Troxler, T.; Yeh, A. G.; Dai, H. L. *Langmuir* **2000**, *16*, 2475. (b) Eckenrode, H. M.; Dai, H. L. *Langmuir* **2004**, *20* (21), 9202. (c) Richmond, G.; Robinson, J. M.; Shannon, V. L. *Prog. Surf. Sci.* **1988**, *28*, 1. (d) Shen, Y. R. *Annu. Rev. Phys. Chem.* **1989**, *40*, 327. (e) Corn, R. M.; Higgins, D. A. *Chem. Rev.* **1994**, *94*, 107. (f) Eienthal, K. B. *Chem. Rev.* **1996**, *96*, 1343.
- (12) Parfitt, G. D.; Rochester, C. H. In *Adsorption from Solution at the Solid/Liquid Interface*; Parfitt, G. D., Rochester, C. H., Eds.; Academic Press: New York, 1983.
- (13) Pereira, M. F. R.; Soares, S. F.; Orfao, J. J. M.; Figueiredo, J. L. *Carbon* **2003**, *41*, 811.
- (14) Braga, M.; Leite, C. A.; Galembeck, F. *Langmuir* **2003**, *19*, 7580.
- (15) Mubarekian, E.; Santore, M. *Langmuir* **1998**, *14*, 1597.
- (16) Giles, C. H.; MacEwan, T. H.; Makhwa, S. N.; Smith, D. J. *Chem. Soc. Abstr.* **1960**, 3973.
- (17) *The Sigma-Aldrich Handbook of Stains, Dyes and Indicators*; Aldrich Chemical Co., Inc.: Milwaukee, WI, 1990.
- (18) Evans, D. F.; Wennerstrom, H. *The Colloidal Domain: Where Physics, Chemistry, Biology and Technology Meet*, 2nd ed.; Wiley-VCH: New York, 1999.
- (19) Lyklema, J.; Overbeek, J. J. *Colloid Sci.* **1961**, *16*, 501.
- (20) Jen, S. H.; Dai, H. L. To be published.
- (21) Boyd, R. W. *Nonlinear Optics*; Academic Press: San Diego, CA, 1992.
- (22) Shi, X.; Borguet, E.; Tarnovsky, A. N.; Eienthal, K. B. *Chem. Phys.* **1996**, *205*, 167.
- (23) Kikteva, T.; Star, D.; Leach, G. W. *J. Phys. Chem. B* **2000**, *104*, 2860.