New Observations at the Air-Water Interface

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Abstract: *The air-water interface is a ubiquitous chemical, physical, and biological entity that is surprisingly poorly understood. Many studies suggest that liquid water at the interface may maintain an ice-like surface structure at temperatures well above its freezing point, though the depth of the structured region is unknown. Polystyrene-latex microspheres in suspension migrate away from the air-water interface forming a possible "exclusion zone." This microsphere-free zone appears to have similar properties to the previously studied exclusion zone that forms next to various hydrophilic surfaces. Reported here are various properties of the exclusion zone at the air-water interface. This region forms regardless of microsphere size, functionalization, or surface charge. Adding solutes decreases the rate of formation of the exclusion zone, but does not necessarily prevent it from forming. The presence of free radicals in solution does not significantly affect exclusion zone formation. Increasing pH of the water can completely prevent the formation of the microsphere-free region at high concentrations of hydroxide ion, but at concentrations less than 1 mM NaOH the hydroxide anions greatly enhance the rate of exclusion zone formation, an effect independent of the cation. Perhaps the most interesting finding at the air-water interface is the "tent phenomenon." This occurs when a capillary tube is touched to the surface of the water above a microsphere-free region forming a shape like the roof of a tent; thousands to millions of water layers beneath the capillary tube, the microspheres are pulled up in exactly the same shape. The fact that the microspheres beneath the microsphere-free region are pulled up suggests that there could be vertical structuring within the microsphere-free region. This, in turn, could provide evidence that the water at the air-water interface is more extensively structured than in bulk. The mechanism for this structuring is largely unknown, and physical properties of this exclusion zone require further elucidation*

1. INTRODUCTION

Interfacial water is relevant in disciplines ranging from nanotechnology to oceanography. Several techniques for studying aqueous interfaces include atomic force microscopy (AFM) [4], optical second-harmonic generation and sum-frequency generation [8], and x-ray and neutron scattering[3]. Higgins et al. [4] used frequency modulation-AFM to measure the number and spacing of water layers next to lipid monolayers, and suggested that 4 to 5 water layers appear to approach the limit of water's structuring ability [4].

There is growing evidence, however, that water next to hydrophilic surfaces exhibits different characteristics from water in bulk [10]. Zheng et al. [10] showed that particles in solution rapidly migrate away from hydrophilic surfaces such as gels and the ionomer Nafion, and suggest that the resultant exclusion zone is a region of more highly structured water. They show that this region has different properties from bulk water, including a strong UV absorbance at 270 nm and a transverse relaxation time (T_2) value different from both the adjacent poly(vinyl alcohol) gel and bulk

water when imaged with nuclear magnetic resonance imaging [10]. These authors rule out a variety of trivial explanations for exclusion zone formation in a previous paper, and show that the exclusion zone forms next to a variety of different types and configurations of gels and is not a peculiarity of a single type of gel [11].

The air-water interface is increasingly studied with techniques such as sum-frequency vibrational spectroscopy and molecular dynamics simulations. Sum-frequency vibrational spectra suggest the coexistence of "icelike" and "liquidlike" hydrogen bonding networks at the air-water interface [8]. This suggests that water at the interface has a partially ordered hydrogen bonding network. Molecular dynamics simulations of the air-water interface have been used especially to determine the distribution of solutes with respect to the interface. These simulations show that large, negative anions tend to be pushed toward the water surface while cations are solvated beneath it [6]. Hydronium ions also are more concentrated at the surface due to their weaker polarity [5]. These several observations suggest that the air-water interface may be

disproportionately affected compared to bulk water by changes in solute concentrations that could affect both surface structuring and surface ion concentrations.

In light of previous work on both the air-water interface and the hydrophilic surface-water interface, we investigated whether the air-water interface forms an exclusion zone similar to that which forms next to hydrophilic surfaces. A simple experiment with a microsphere-water suspension in a capillary tube showed that microspheres seem to translate away from the surface of air bubbles positioned at both ends of the tube, leaving microsphere-free regions at the air-water interface. In this paper, we sought to determine both if an extensive exclusion zone forms at the air-water interface, and also what factors might affect the formation of such an exclusion zone. We present evidence that the exclusion zone, hereafter referred to as the microsphere-free region (MFR), that forms at the air-water interface can be affected by the addition of ions to solution and the size of the particles put into suspension, and may represent a region of more highly structured water than bulk water.

2. MATERIALS AND METHODS

Studies of the effects of solutes on the formation of a microsphere-free region at the air-water interface were completed using custom made glass chambers of dimensions 76 x 25 x 3 mm. 1 or 2 μm carboxylate- or amino-functionalized microspheres (Polysciences, Inc., Warrington, PA) were used to visualize differences between bulk and interfacial water. Type I water (NANOpure DIamond, Barnstead International, Dubuque, IA) was used to make solutions for all experiments. Microsphere suspensions (0.025% solids) were made immediately before addition to the glass chambers; after addition of 4 ml of suspension, the chambers were sealed with Parafilm. We found no significant difference in behavior of the interfacial region when different concentrations of microspheres were used (Figure 1). We found that the MFRs at the air-water interface formed most uniformly and quickly when chambers containing microsphere suspension had parafilm applied to the surface. If not covered with parafilm, the chambers

tended to form "sinkholes," regions where the microsphere-free layer appears to have fallen down to create a clear column, rather than a uniform MFR. All experiments took place at room temperature under ambient light conditions unless otherwise noted. Images of these chambers were taken at intervals with a Kodak P880 digital camera. Image J (NIH) was used to analyze the size of the microsphere-free region.

Experiments exploring the effects of various chemicals on the formation of the MFR were conducted using laboratory grade chemicals diluted to the desired concentration immediately before the experiment took place.

For oil-water interface experiments, chambers were set up as above and silicon diffusion pump oil (Dow Corning, Midland, MI) was pipetted onto the surface until it covered the water. This layer was approximately 1 mm deep. Images were taken every 15 min to monitor the rate of formation of the MFR at the oil-water interface compared to the air-water interface.

Light intensity experiments were conducted at ambient temperature. A 2 μm carboxylate-functionalized microsphere suspension that was 0.025% (vol/vol) solids was added to two chambers which were then sealed with parafilm. One chamber was left under ambient light conditions while the other was positioned under a standard gooseneck fiber optic lamp and illuminated with high-intensity white light. For the experiment determining the effect of heat on the formation of this zone, two chambers were prepared in the same way as for the light experiments. One chamber was left on the benchtop at ambient light and heat while the other was placed on a heating block set to 35ºC under ambient light conditions and both chambers were monitored over time.

After a microsphere-free region had formed, if a glass capillary tube (either open or closed) was touched to the surface of the water, both the microsphere-free surface water and the microsphere containing water beneath were pulled up due to capillary action. Movies showing the lateral motion of the "tent phenomenon" were taken with a CCD camera (Scion Corporation, Frederick, MD) using Image J software. Images were captured every second as a mounted capillary tube was moved across the water surface. Movies showing the effects of

vibrating the capillary tube on the MFR were captured either with a Kodak P880 digital camera or a Scion CCD camera mounted on a microscope. The capillary tube was mounted on

a speaker with a function generator which allowed for modulation of frequency and amplitude of vertical vibrations of the capillary tube.
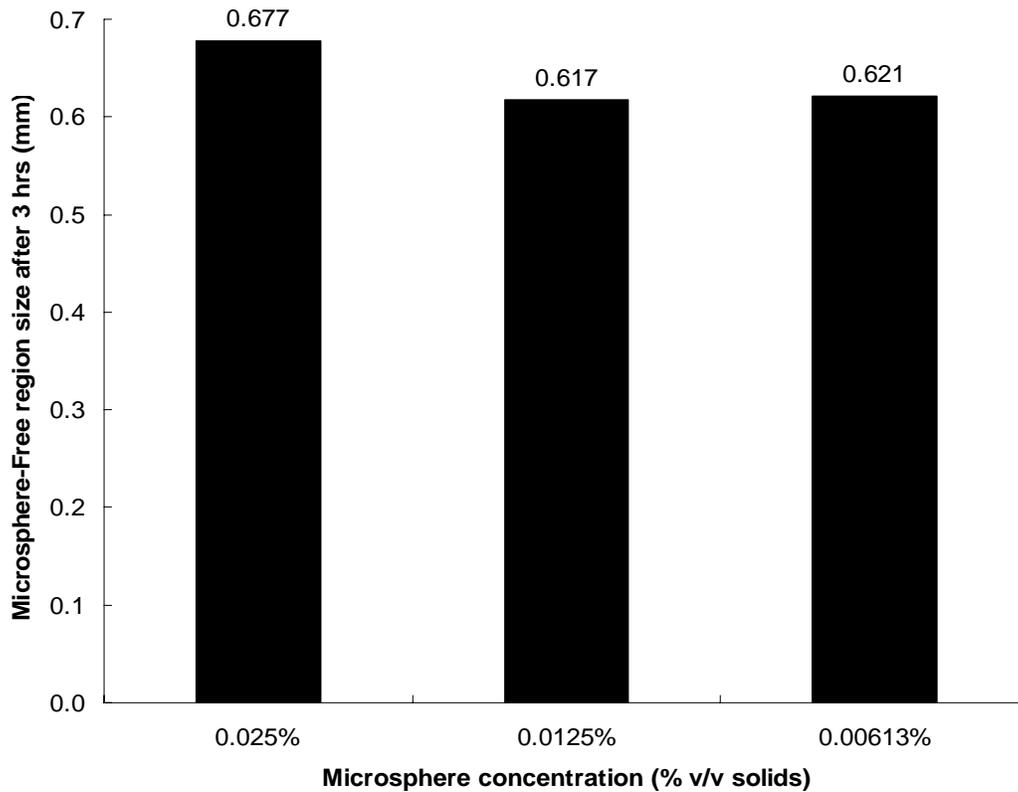

Figure 1. *Size of microsphere-free region after 3 hours with different concentrations of microspheres in suspension. Though this experiment was not repeated, the difference between the size of the microsphere-free region (MFR) with different concentrations of microspheres was less than 0.06 mm and therefore was not considered significant for our purposes.*

Optical microscopy using thin chambers was preformed using a microscope oriented so that a slide could be viewed vertically because the optical axis of the microscope was parallel to the earth. Small chambers < 1 mm wide composed of glass cover slips mounted on a spacer were used to study small bodies of water. These chambers could be flipped on their sides or upside-down so that the air-water interface was not always above the bulk water, and thus water structuring at the air-water interface could be explored against the force of gravity. Samples

were illuminated with a green LED and images were captured with a CCD camera via Image J.

3. RESULTS

3.1 Effect of chloride salts

In order to determine the effects of salt on exclusion zone formation, microsphere suspensions were made with 2 μm carboxylate-coated microspheres and several concentrations of NaCl: 0 mM, 0.5 mM, 10 mM, and 100 mM NaCl. After 4 hr, the size of the MFR for the distilled water suspension was 2.33 mm while
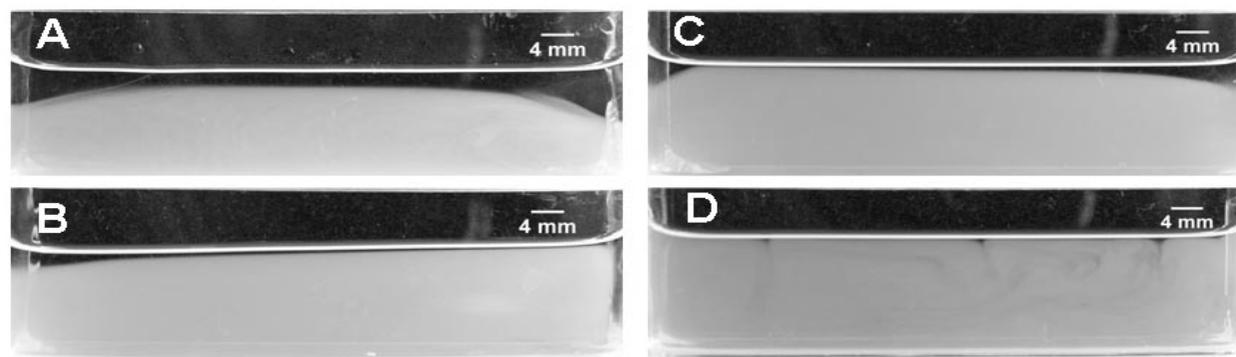
Figure 2. *Microsphere-free region in various [NaCl] solutions. (A) Deionized water, (B) 0.5 mM NaCl, (C) 10 mM NaCl, (D) 100 mM NaCl. All chambers contained suspensions with 2 um carboxylate-functionalized microspheres at a concentration of 0.025% v/v solids. Images are taken 4 hours after addition of microspheres to chambers.*

the MFR for the 0.5 mM NaCl microsphere suspension was only 0.75 mm and that for the 100 mM NaCl suspension was 0.25 mm (Figure 2). The 100 mM NaCl suspension was more likely than others to form a series of small sinkholes rather than an obvious exclusion zone. To determine if the presence of a salt affected MFR formation for microspheres with different surface functionalizations, an experiment was set up with six variables. Three 100 mM salt solutions (NaCl, LiCl, and KCl) had either 1 μm amino-, 1 μm carboxylate-, or 2 μm carboxylate-functionalized microspheres added to them. At the pH of this experiment (~7), the amino-functionalized microspheres should be protonated and thus have a positive charge, while the carboxylate-functionalized microspheres are deprotonated and have a negative charge. For 1 μm microspheres, a MFR is not readily visible with the naked eye for approximately 6 hr regardless of functionalization. 1 μm carboxylate- and amino-functionalized microspheres in 100 mM salt solutions show little/no formation of the MFR after those suspensions in deionized water have a visible MFR (not shown). 2 μm microspheres in salt solution do not readily form a uniform MFR but rather form vertical patterns much like sinkholes previously mentioned in Materials and Methods.

3.2 Effect of Ph

Previous studies showed that the exclusion zones observed next to most hydrophilic surfaces are negatively charged in deionized water [10]. One suggestion is that negative ions

could be instrumental in forming this region. To try to elucidate this further, we changed the pH of the microsphere/water suspension to determine if this has an effect on the MFR. The effect of pH on the formation of the MFR was observed for the three microsphere sizes/functionalizations listed above. Even highly acidified microsphere suspensions (pH < 1) showed fairly little difference between the controls in deionized water (not shown); however, for basic solutions pH has a large effect on MFR formation. When the concentration of hydroxide anions is on the order of 50 μM, MFR formation is enhanced compared to controls (Figure 3A), but when the hydroxide concentration is greater than 1 mM, MFR formation is essentially completely inhibited (Figure 3B). Between the hydroxide ion concentrations of 50 μM and 1 mM, there was a clear MFR but it takes on an unexpected wavy shape (Figure 3B). The inhibition of MFR formation by high concentrations of hydroxide anions is reminiscent of high salt concentrations, but does not look exactly the same. Additionally, to determine if some pH gradient exists in water that has formed an MFR, low concentrations of pH dye (Sigma Aldrich) were added to microsphere suspensions to determine if MFR formation coincided with the generation of a pH gradient. No pH gradient was visually detectable with this method, however, so no conclusion can be drawn about whether the MFR has different concentrations of protons or hydroxide anions than the bulk water (not shown). Additionally, we could not detect any kind of dye exclusion at the air-water interface with our methods. No

difference in pH was observed between water taken from the MFR and bulk water.

3.3 Effect of free radicals

Another hypothesis about formation of the MFR or the exclusion zone next to hydrophilic surfaces that we wanted to test was that the MFR requires free radicals to help structure the water within it. This hypothesis was tested by adding small amounts of either ascorbic acid (a free radical sequestering agent) or hydrogen peroxide (free radical producing agent). Addition of both hydrogen peroxide (0.3%) and ascorbic acid (500 µM) slightly reduced the rate of formation of the MFR for all three types of microspheres (not shown), but these results are probably not significant. Concentrations of ascorbic acid below 500 µM appeared to have no effect (not shown).

3.4 Tent phenomenon

An important result of this work is that the MFR at the air-water interface exhibits a property we call the "tent phenomenon." This occurs when a capillary tube is touched to the surface of the water above an MFR forming a tent shape, and, thousands to millions of water layers beneath the capillary tube, the microsphere-containing water is pulled up to form exactly the same shape as the surface. Though this phenomenon is most striking when the MFR is small, microspheres can be visibly affected when a capillary tube touches the surface when the MFR is as large as 2 mm (Figure 4). When the capillary tube is moved up and down or across the surface of the water, the microsphere "tent" beneath it moves in the same way (Figure 4B). When the capillary tube was moved up and down with low (~0.5 mm) amplitude vibrations, however, the MFR could be destroyed if less than 1 mm deep. When amplitude of the vibrations was increased, the MFR changed shape to form a more wave-like barrier between the clear region and the microsphere-containing water suspension (not shown).
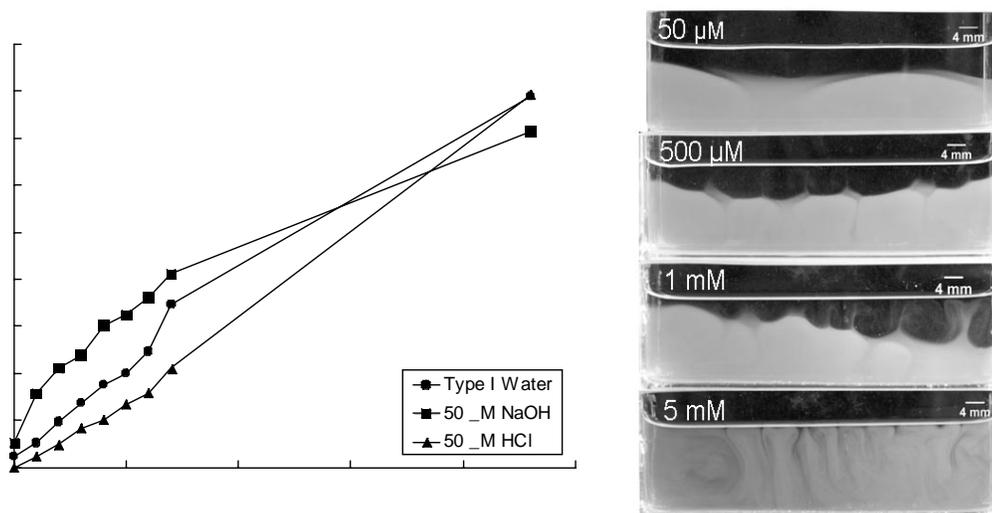

Figure 3. *Change in size of microsphere-free region over time for deionized water, 50 µM NaOH and 50 µM HCl solutions with 2 µm carboxylate-functionalizedmicrospheres. B) Images of microsphere-free region in chambers with variousconcentrations of NaOH at t=5 hr after addition of microsphere suspension to chambers.*
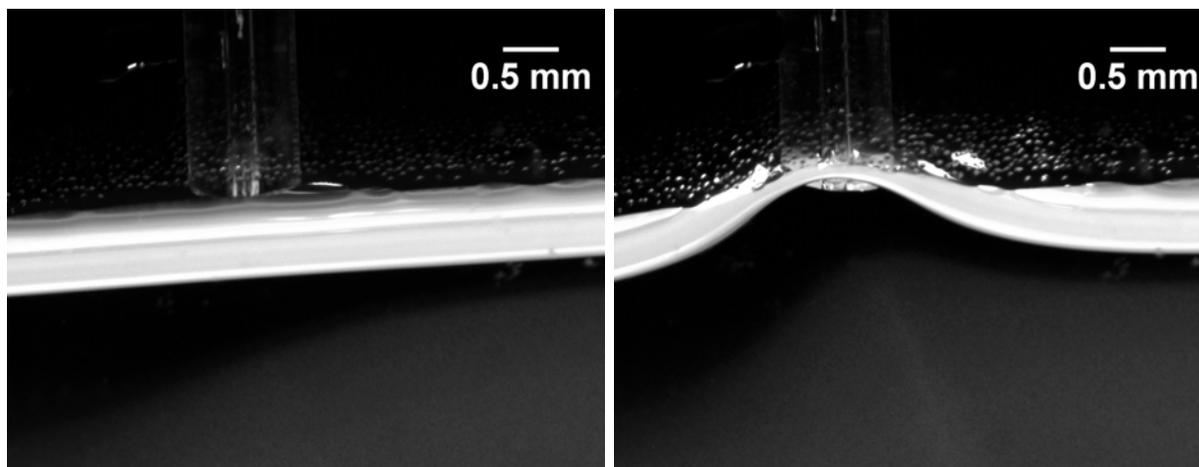
Figure 4. *Tent phenomenon occurs when a capillary tube is touched to the surface (bright) of the microsphere-free region which appears black, and the microspheres (white) thousands or millions of water layers beneath it are pulled up in the same shape as the water surface. Arrows point to relevant change of shape of microsphere-containing region.*

3.5 Air-water versus oil water interfaces

To determine if sedimentation, rather than interfacial interactions, is responsible for the MFR, silicon oil was applied to the surface of the water-microsphere suspension and the rate of formation of the MFR was monitored over time and was compared to a control of a microsphere suspension without oil (Figure 5). This controls for sedimentation because microspheres in suspension would sediment equally quickly no matter if the interface were covered in oil; however, if the air-water interface caused MFR formation, covering the interface with oil should prevent or at least slow MFR formation. This experiment was run three times at different times of day. Though for each individual trial the MFR formed slower at the oil-water interface than the air-water interface, the results of multiple trials yielded no significant difference after about an hour. However, it seems possible that the error bars could be reduced if the experiment were run more times, potentially revealing a significant difference. Potential factors that varied across trials that could affect the formation of the MFR include slight differences in temperature and light intensity. In order to determine if these variables played any role, a chamber was

illuminated with bright white light while a control chamber was left on the bench with only ambient light. The chamber that was highly illuminated never formed a clear MFR over a period of >6 hr while the control chamber did form an MFR. Similarly, when a chamber heated from beneath on a hot plate was compared to a control chamber on the benchtop, the heated chamber formed no MFR over the course of the > 6 hr experiment while that on the benchtop did form a distinct MFR. Only one trial of each of these experiments was conducted, so further work would need to work out the mechanism behind the inhibitory effects of temperature or light on the MFR.

3.6 Rotated orientation of air-water interface

To further elucidate whether the macroscopic MFR observed at the air-water interface is due to gravitational effects (sedimentation) of microspheres, small volumes of microsphere suspension were examined under a microscope in narrow chambers. These chambers were thin enough that capillary action could hold the water in the chambers even when the chambers were flipped upside-down. In this way, the air-water
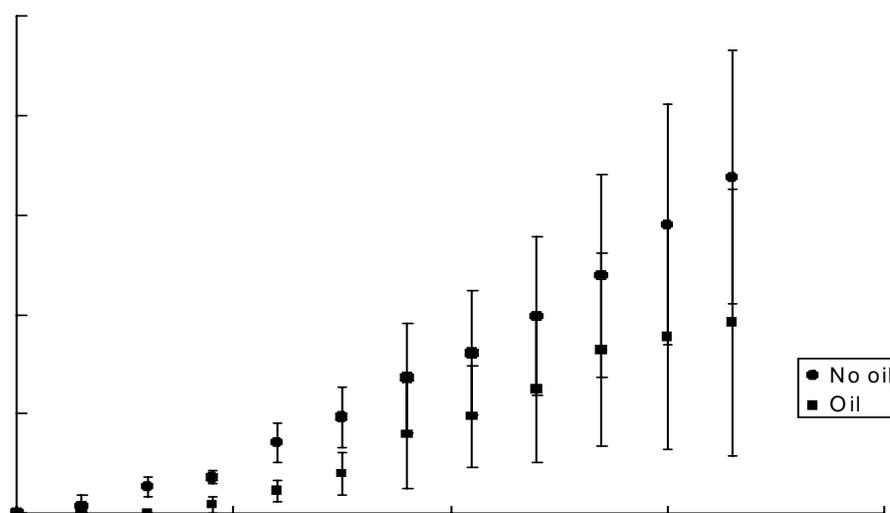
Figure 5. *Growth of microsphere-free region over time in deionized water with 2 μm carboxylate microspheres. Squares represent chambers in which mineral oil covered the surface of the water to create an oil-water interface rather than an air-water interface. Circles represent chambers with no oil on the surface. Error bars represent the standard deviation of three trials. Trials were conducted on different days, so atmospheric differences may have contributed to lack of significant difference between the two*

interface could be explored in diverse orientations so that the air did not necessarily have to be above the water. A time-lapse video of the air-water interface next to a bubble in the chamber showed that after approximately 15 min, a distinct MFR formed next to the bubble and after 25 min it grew to 40 μm wide (not shown). This and similar experiments were repeated a number of times, but failed to yield conclusive results because sometimes a clear MFR formed at the interface, and other times it failed to form within the same time-frame. It is therefore unclear how to interpret these results, though it is possible that factors such as relative humidity and evaporation could affect this region.

4. DISCUSSION

4.1 Salts

Experiments with various concentrations of several chloride salts in solution revealed a strong negative correlation between the size of the microsphere-free region and the concentration of salt. For example, the size of the MFR in 100mM NaCl was smaller than that

in deionized water by a factor of 9. This suggests that electrostatic interactions may be involved with exclusion zone formation because when salts are added, ions in solution may shield lower water layers from any charge acquired at the surface. Another hypothesis is that since the concentration of halides in water is enhanced at the air-water interface compared to bulk [2], the chloride ions may inhibit some interaction between air and water that perhaps causes water molecules to orient a particular way or gives the surface some charge.

4.2 pH

Changing the pH of the microsphere suspensions radically changed how the microspheres translated away from the air-water interface. We had previously hypothesized that hydroxide ions are essential to the formation of the MFR because the analogous region previously documented next to hydrophilic surfaces [10] has a negative charge, which we attribute to an excess of OH^- ions. We therefore supposed that adding base to the microsphere suspension would increase the rate of formation of the MFR because the excess hydroxide ions would be

present to "build" this region. However, we found that too large an excess of these ions actually completely inhibited formation of the MFR while lower concentrations of hydroxide ions enhanced its formation. Protons (H^+) appeared to slightly decrease the size of the MFR, but this effect was not strictly concentration dependent. These data appear to imply that up to a certain concentration, hydroxide ions do play some role in the formation of the MFR, but at greater concentrations there could be some stronger electrostatic interference taking place, perhaps due to the sodium counterions, that overwhelms the effect of the hydroxide ion excess. Electrical potential measurements of the air-water interface have so far proven inconclusive, ranging from +0.5 V [1] to -1.1 V [9], which makes these conclusions somewhat speculative. Some molecular dynamics and spectroscopy evidence suggests that hydroxide ions are weakly excluded from the interface while hydronium ions are weakly accumulated [7]. Since negative halide ions are also accumulated at the interface, it is possible that an excess of any charged bodies at the interfaces somehow affects the formation of the MFR. Another hypothesis is that ions in solution affect the interactions between microspheres and alter the properties of the colloidal suspension, perhaps changing some properties that affect sedimentation rate which is visually manifest in a change in the size and rate of formation of the MFR.

4.3 Free radicals

Experiments reported here failed to show any connection between the presence of free radicals in solution and the formation of the microsphere-free region, since both the free radical producing agent and the free radical sequestering agent had the same effect on the MFR. It seems more likely that the presence of any solutes in solution decreases the rate of formation and ultimate size of this zone. This, again, could be due to electrostatic interactions or changes in the interactions between microspheres in suspension.

4.4 Oil-Water vs Air-Water interface

The experiment described above which attempted to determine if the MFR at the air-water interface forms differently from that at the oil-water interface was somewhat inconclusive, though more trials could reveal a statistically significant result. This result is probably due to variability in light intensity or temperature between the various days that the trials were conducted. This suggests that temperature or light intensity may be a major factor in the formation of the microsphere-free interfacial region. Experiments where these variables were manipulated suggest that high light intensity, high temperatures, or both negatively affect the formation of the MFR. It is possible that the elevated heat or light intensity provide excess energy that disturbs any ordering the water molecules in this region may have.

4.5 Tent Phenomenon

Perhaps the most important experiment presented here, the tent phenomenon, provides some evidence for possible vertical water structuring in the MFR. The microsphere-containing region lies beneath the clear MFR. When a capillary tube is touched to the surface of the water above the MFR, this surface water is pulled up due to capillary action. The fact that the microsphere-containing region beneath is also pulled up in the same way as the surface may suggest that the MFR is structured, thus linking the water at the surface with water many layers beneath it. Thus, vertical structuring may explain why a region thousands to millions of water layers beneath the surface forms the same shape as the surface region.

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