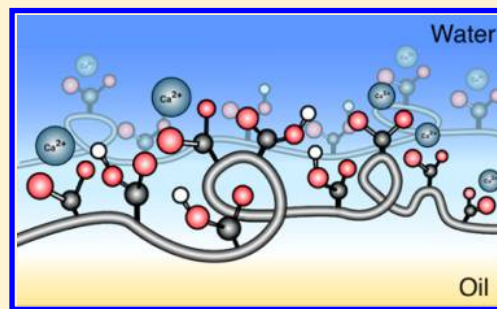


Metal Ions: Driving the Orderly Assembly of Polyelectrolytes at a Hydrophobic Surface

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ABSTRACT: The accumulation of polyelectrolytes at the interface between water and nonpolar fluids is an important process in both environmental and biological systems. For instance, polyelectrolytes such as humic acids are highly charged molecules that play a role in the remediation of water contaminated by oil, and the adsorption of other polyelectrolytes such as proteins and DNA to cellular surfaces is essential in biological processes. The properties of these naturally occurring polyelectrolytes are highly tunable and depend strongly on the binding of metal ions commonly found in environmental and biological systems. While the metal complexation behaviors of many polyelectrolytes and biomolecules are well characterized in bulk solution, this work shows in molecular detail that the behavior of a common polyelectrolyte in the presence of metal ions can be quite different when it adsorbs to a hydrophobic–aqueous liquid interface. In these studies, vibrational sum frequency spectroscopy and interfacial tension measurements conducted on poly(acrylic acid) (PAA) at a model oil–water interface show how small amounts of monovalent and divalent cations significantly alter the interfacial conformation of PAA at the interface and act to enhance its interfacial adsorption. The results provide important new insights that have direct relevance for understanding the effect of metal ions on the adsorption of charged macromolecules to a hydrophobic–aqueous boundary layer, specifically in biological and environmental systems.



INTRODUCTION

Polyelectrolytes (PEs) have captivated the interest of scientists for many decades and have been studied in great detail, as evidenced by the quantity of literature on the subject. They have a wide variety of applications in industrial settings, such as oil recovery and cleanup,^{1–3} water remediation,^{2,4–7} electrical multilayer assemblies,^{8–10} complexation with nanoparticles,^{11,12} fabrication of microwires,¹³ as well as a number of biological applications.^{14–17} Notably, simple PEs are similar to a number of naturally occurring molecules in environmental^{18–21} and biological²² systems, and thus can act as molecular models for studying these complex systems in controlled experiments. Poly(acrylic acid) (PAA), the focus of this study, is a simple PE with particular relevance as a model for humic substances and biological macromolecules because of its single carboxylic acid repeat unit. The equilibrium between a carboxylic acid and its anion is pH sensitive, and the acid/anion ratio strongly affects the conformation of macromolecules in aqueous solution,^{23–25} their solubility,²⁶ and their ability to go to interfaces.²⁷ Although there have been extensive studies of PEs and PAA in bulk solution^{28,29} and adsorbed to both planar solids^{30–32} and nanoparticle surfaces,^{33–35} very few studies have been able to provide molecular level information about how these interesting macromolecules adsorb at liquid interfaces where their applications are often most relevant in biological and environmental systems.

Beyond controlling the amount of charge on weak PEs and dictating their bulk structural characteristics, the carboxylate groups on these PEs strongly interact with metal ions.^{36,37} For example, humic substances are known to strongly bind both

toxic and nutritious metals for transport through soils and into water sheds,^{20,21} and there are several types of proteins that are strong metal binders.³⁸ These macromolecular–metal interactions are well-known to affect PE conformation and functionality in bulk solution.^{38–40} The effect of metals ions on the behavior of PEs at fluid interfaces is largely unknown. Such information is important as PEs can often reside at a junction where such binding to the metal can affect transport across a fluid interface and solubility of the macromolecule.⁴¹

To probe the adsorption of PAA at a liquid–liquid interface and the effect of metal ions on this adsorption, we use vibrational sum frequency (VSF) spectroscopy and interfacial tensiometry. In our initial investigations of PAA adsorbing to the CCl₄–water interface, we found remarkable sensitivity in the adsorption process to the aqueous phase pH.^{42,43} The pH studies suggested that the adsorption is a multistep process that depends strongly on PAA charge density, a result that is further supported and clarified in this Article. In the course of these further studies, we have discovered that this very interesting multistep adsorption process and strong pH dependence are also significantly affected by the presence of aqueous phase ions that can alter the electrostatics and conformation of the polymer, in some cases driving the polymer to the interface under conditions where adsorption otherwise was prevented. The results reported here for the monovalent (NaCl) and divalent (CaCl₂ and MgCl₂) metal ions have important

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implications for interfacial processes of importance in biological and environmental sciences, specifically in the case of the placement of toxins and nutrients within the environment.

■ EXPERIMENTAL DETAILS

Interfacial Tension. Interfacial tension experiments were carried out on a KSV optical tensiometer using the pendant drop method. Interfaces were prepared by filling a cuvette with CCl_4 and forming a drop by inserting the hooked needle of a syringe containing the aqueous solution into the cuvette. Data acquisition was started upon formation of the drop. When acquiring data of aqueous polymer solutions, a neat CCl_4 –water interface was always prepared first, and the interfacial tension value was verified against the known value of CCl_4 –water, which is 44 mN/m.^{44,45} Once the correct neat interfacial tension value was achieved, the syringe was filled with the polymer solution of interest and a pendant drop was made. Data were recorded until an equilibrium interfacial tension value was achieved, or in the cases of the long time acquisitions, there was drop failure. Interfacial tension values were calculated from the acquired images using the KSV software. Surface tension was calculated by first using the Young–Laplace equation to find the shape factor, β , and the following equation:

$$\gamma = \frac{\Delta\rho g R_0^2}{\beta} \quad (1)$$

This relates the surface tension (γ) to the drop shape through the difference in the density of the fluids at the interface ($\Delta\rho$), the gravitational constant (g), the radius of the drop curvature at the apex (R_0), and the shape factor (β).

Spectroscopic Measurements. VSF spectroscopy data were acquired with an Ekspla laser and IR generation system with adaptations to accommodate the liquid–liquid cell and inverted beam geometries. This system has been described in detail elsewhere.⁴⁶ Briefly, a YAG laser outputs 1064 nm pulsed light with an ~ 30 ps pulse length. The 1064 nm light is split into two lines, and one line is frequency doubled to give 532 nm light. A small portion of the 532 nm line is used as the visible portion at the interface, while the remainder of the 532 nm line and the 1064 nm line are used to generate tunable infrared light via an OPG/OPA/DFG setup. Using a total internal reflection (TIR) geometry, the incident beams pass through the CCl_4 at their respective TIR angles. CCl_4 is used as the organic solvent because of its transparency in the infrared region of interest.

The intensity of the detected sum frequency is proportional to the square of the effective second-order susceptibility $\chi_{\text{eff}}^{(2)}$ and the intensity of the incident IR and visible beams as shown in the following equation:

$$I(\omega_{\text{sf}}) \propto |\chi_{\text{eff}}^{(2)}|^2 I(\omega_{\text{vis}}) I(\omega_{\text{IR}})$$

The second-order susceptibility, $\chi^{(2)}$, and the effective second-order susceptibility from the previous equation are related through the Fresnel coefficients and unit polarization vectors. $\chi^{(2)}$ is composed of a nonresonant component and the sum of all of the resonant components as shown in the following equation:

$$\chi^{(2)} = \chi_{\text{NR}}^{(2)} + \sum_{\nu} \chi_{R_{\nu}}^{(2)} \quad (3)$$

The resonant second-order susceptibility, $\chi_{R_{\nu}}^{(2)}$, is as follows and is dependent on both the number density of the molecules at the interface as well as the molecular hyperpolarizability, β .

$$\chi_{R_{\nu}}^{(2)} = \frac{N}{\epsilon_0} \langle \beta \rangle \quad (4)$$

The angled brackets around β indicate that this is an orientational average over the probed macroscopic system. The dependence of the sum frequency signal on both the number density at the interface and the molecular orientation allows for a thorough understanding of

interfacial adsorption. In addition, the polarization of the generated sum frequency signal is dependent on the incident polarizations of the visible and IR beams. In this study, all spectra are in the ssp polarization scheme (unless otherwise noted), which denotes the polarization of the sum frequency, visible, and IR beams, respectively. This polarization scheme probes vibrational modes that have dipole components that are normal to the plane of the interface.

Normalization of spectra is required to take into account changes in overlap, timing, and IR energy across the spectral window. Spectra in the water region were normalized by dividing the spectra by the IR energy. Normalization in the mid-IR region required dividing by a nonresonant gold spectrum to eliminate an artifact in the IR profile in this region. Spectral fitting is accomplished using the expression shown below, which accounts for both homogeneous and inhomogeneous broadening.^{47,48}

$$|\chi^{(2)}(\omega_{\text{SF}})|^2 = \left| \chi_{\text{NR}}^{(2)} + \int_{-\infty}^{\infty} \frac{A_{\nu} e^{-[(\omega_{\text{L}} - \omega_{\nu})/\Gamma_{\nu}]^2}}{\omega_{\text{L}} - \omega_{\nu} - i\Gamma_{\nu}} d\omega_{\text{L}} \right|^2 \quad (5)$$

Sample Preparation. Chemicals were purchased in the highest purity possible from Sigma-Aldrich (PAA, NaOH 1.0 N solution in water, HCl 37%, CaCl_2 , MgCl_2 , NaCl). Metal ions were specifically chosen to have a common counterion (chloride) to prevent any ambiguous counterion effects at the interface. Solutions were prepared using clean glassware, an analytical balance, and water from a Barnstead Nanopure II system. Solution pH was adjusted using NaOH or HCl and tested using EMD pH paper with regular verification via an Oakton 110 series pH meter.

The sample cell was machined from a solid piece of Kel-f with two windows set normal to the incident and outgoing 532 nm beam. Windows are sealed with DuPont Kalrez perfluoropolymer O-rings. The input window was CaF_2 , and the output window was BK-7 glass. All glassware, the cell, the BK-7 window, and the O-rings were soaked in concentrated sulfuric acid with No-Chromix for a minimum of 12 h, and then each piece was rinsed with 18 M Ω water from a Barnstead ePure filtration system for at least 25 min. The CaF_2 window was allowed to soak in the same acidic solution for 15–20 min and then thoroughly rinsed.

Data acquisition started immediately after the interface was prepared. Each VSF spectrum shown in these experiments is an average of at least 400 laser shots per data point. Long-term equilibration was checked by letting the interface sit for ~ 6 h and then retaking the spectrum. Unless otherwise noted, equilibration of the VSF signal was reached almost immediately.

■ RESULTS AND DISCUSSION

Adsorption of PAA to the Oil–Water Interface. As background for the studies of the effect of polymer concentration and metal ion complexation, this section begins with a brief description of our recently derived understanding of how PAA adsorbs to the CCl_4 –water interface, highlighting the roles of pH and molecular weight. Figure 1 shows a VSF spectrum and interfacial tension data (inset) for PAA (5 ppm, 450 kD, pH 2) at the CCl_4 –water interface. The VSF spectrum from 1275 to 1900 cm^{-1} shows a single peak at 1732 cm^{-1} , which is assigned to the carbonyl stretch mode of the carboxylic acid on the polymer. The single intense carbonyl peak in this initial spectrum indicates that polyelectrolyte is present at the oil–water interface and that its carboxylic acid modes are highly oriented perpendicular to the interface. Spectra taken in the C–H stretch region (2800–3000 cm^{-1}) also show strong C–H mode alignment, indicating that the polymer backbone also has a high degree of alignment perpendicular to the interface. The steady decrease in interfacial tension, until an equilibrium value of ~ 31 mN/m is reached after ~ 1200 s, supports the picture of polymer at the interface (Figure 1, inset).

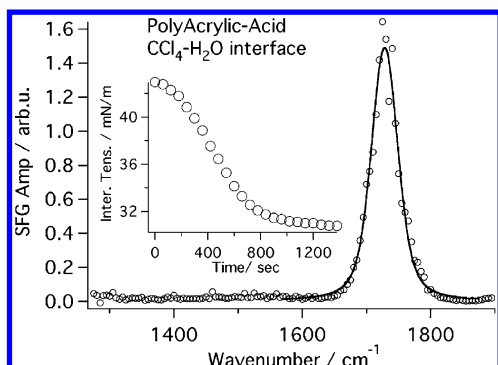


Figure 1. VSF spectrum (ssp polarization) of PAA (5 ppm, 450 kD, pH 2) at the CCl₄–water interface showing both the carboxylate and the carbonyl stretch regions. The solid line is a fit to the peak at 1732 cm⁻¹. The inset is an interfacial tension measurement of the same system using the pendant drop method.

Previous work in this laboratory showed significant pH dependence for the adsorption of PAA to the oil–water interface.⁴² A sharp transition point at pH 4.5 was discovered, which defined the point at which PAA adsorption (below pH 4.5) or desorption (at and above pH 4.5) occurred. The VSF spectra showed no variation with pH below 4.5, indicative of no variation in the adsorbed layer under these more acidic conditions. At pH 4.5 and above, neither the VSF spectroscopy nor the interfacial tension measurements showed any polymer presence at the interface. This transition pH is much lower than the pK_a of PAA in bulk (~6).^{49–51} This difference was attributed to cooperative processes that allow charges to accumulate near each other on the polymer chains,²³ making it more energetically favorable for desorption to occur at a pH lower than the bulk pK_a.

The VSF spectroscopy and interfacial tension data also showed interesting differences in adsorption dynamics. While the spectroscopic data showed no time dependence, with maximum VSF signal for both the carbonyl and the C–H modes being obtained instantaneously after interface preparation, the interfacial tension measurements showed an equilibrium time of ~20 min.

It was thus concluded that the initial adsorption of PAA occurred very fast with highly oriented carboxylic acid and methylene groups along the backbone. This fast adsorption step has also been seen in interfacial tension studies of biological charged polymers at the air–water interface and was attributed to the rapid adsorption and uncoiling of polymer chains into flat configurations at the interface.^{52,53} The very small change in interfacial tension values seen in these cited studies was

attributed to the loss in entropy associated with the change in polymer conformation as it adsorbed to the interface.⁵² This same analysis is consistent with our observations in the present study. The initial adsorption and spreading of PAA at the interface occurs so quickly we simply do not detect it with sum frequency experiments. Despite the loss in entropy associated with the post-adsorption uncoiling, this two-part process remains energetically favorable due to both the hydrophobic interactions between the alkyl backbone and the oil phase as well as the inherent electric field at the oil–water interface that acts to induce both the rapid adsorption and the orientation of PAA.

Again, VSF showed no time dependence, but the interfacial tension measurements in this study demonstrated a continuous adsorption of PAA to the oil–water interface. As the VSF spectra showed no change with this continued buildup of polymer, it was concluded that late-arriving PAA adsorbed with no net molecular orientation, in a randomly coiled configuration. This is likely due in part to the inability of the electric field at the interface to penetrate deep enough into the water phase to affect these late-arriving polymer chains. In any event, if there were any net orientation in the layers of late-arriving polymer, we would have expected to see an increase in VSF intensity of the CH₂ backbone modes with time. The molecular weight of the adsorbing PE was found to play no role in the initial fast adsorption of ordered polymer, but it played a significant role in the equilibrium dynamics for the slower unstructured adsorbing polymer, indicating that the adsorption rate was controlled primarily by diffusion.⁴³

Effect of PAA Concentration on Adsorption Dynamics. To explore these temporal results further, VSF spectroscopy and interfacial tension measurements were conducted for 450 kD PAA as a function of bulk concentration at the CCl₄–water interface. Each solution was adjusted to pH 2, which ensures that the polymer is in the protonated/neutral state for the experiments. The interfacial tension measurements (Figure 2) show that PAA at a concentration of 50 ppm reaches equilibrium almost immediately, whereas a lower concentration of 5 ppm requires ~1000 s. At 4 ppm, the absorption equilibrium is not reached until ~1500 s. At a concentration of 1 ppm PAA, the time required to reach equilibrium is greater than 4 h.

All solutions approach very similar interfacial tension values, indicating that the equilibrium surface coverage is similar for differing concentrations, even though a factor of ~50 exists between the lowest and highest concentrations shown in Figure 2. In addition, the time required to achieve surface coverage varies by a factor of ~400 between the fastest and slowest time

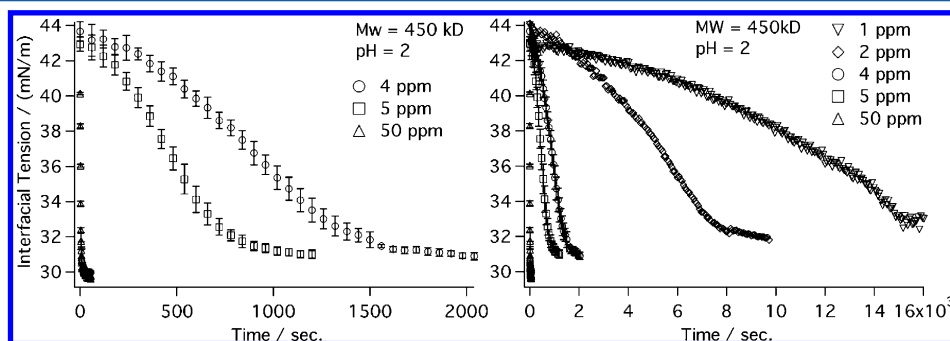


Figure 2. Interfacial tension of PAA as a function of time for five different concentrations.

to equilibrium in Figure 2. This shows that the bulk concentration has a large effect on adsorption time, but little effect on the equilibrium surface coverage, providing further support that adsorption of the polymer to the interface is governed primarily by diffusion. In contrast, the VSF spectra in the carbonyl and C–H stretch regions show no variation with concentration or with time except at very low concentrations. Below 1 ppm, the interfacial tension shows the adsorption process slows to the order of hours and there is a corresponding increase in the intensity of the carbonyl stretch mode as the interface becomes populated. Figure 3 shows VSF spectra of 0.25 ppm PAA at the CCl_4 –water interface over this long equilibration time.

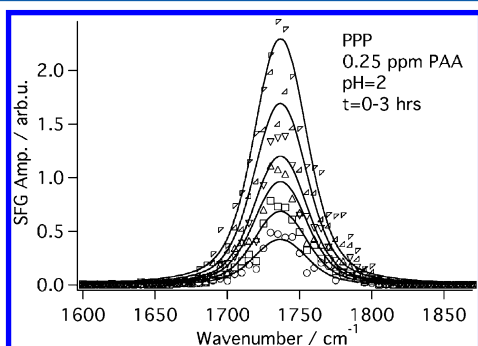


Figure 3. VSF spectra (ppp polarization) of 0.25 ppm PAA at the CCl_4 –water interface taken in ~ 20 min intervals over a 2 h time frame after introduction of PAA.

The solid lines in the Figure 3 are fits from a global routine designed to fit the entire time series to a single peak at 1732 cm^{-1} while holding all parameters constant except for the amplitude. The spectra were first fit individually, and all fit parameters except the amplitudes were found to be identical within experimental error. The ppp polarization scheme was used in this experiment instead of spp as it gives a more comprehensive measure of all oriented material in the interfacial region, not just those modes having a dipole component normal to the plane of the interface, and thus provides a more thorough understanding of PAA adsorption dynamics rather than specific orientational information. Over a period of 2 h a steady increase in signal is observed with maximum signal achieved after approximately 3 h. The surface tension lowers slightly over this time frame and does not reach an equilibrium value for many hours beyond. In contrast, for the slightly higher concentration of 1 ppm, the carbonyl mode of Figure 3 is measured within a few minutes and is constant beyond that time, whereas the surface tension shown in Figure 2 indicates that PAA continues to adsorb for up to 4 h longer. The VSF spectra of these very low PAA concentrations indicate that the adsorption of this first layer is limited by diffusion of PAA to the interface. The combined concentration results are supportive of the earlier derived picture of an adsorption process where an initial ordered layer diffuses to, adsorbs to, and uncoils at the interface with subsequent layers adsorbing but without the ordered orientation of the initial layer.

This multistep adsorption process raises the interesting question of why and how the polymer continues to adsorb to the interface after its initial layer formation. Clearly, the slower adsorption must be energetically favorable and is most likely due to hydrophobic interactions between the polymer chains. Unlike the initial layer, where a decrease in entropy is

compensated for, in part, by the electric field at the interface that drives the orientation of the carbonyl groups and polymer backbone, later arriving chains do not achieve this ordering and therefore can take different conformations to both maximize hydrophobic interactions between polymer chains and increase the entropy of the system.

Strong evidence for such behavior can be seen in our previous studies of different isomers of poly(methacrylic acid) (PMA) at the oil–water interface.⁴³ In these studies, it was shown that for both isotactic PMA and atactic PMA, the initial arriving polymer chains that adsorb within seconds to the oil–water interface are highly oriented, as observed spectroscopically through both the CH and the carbonyl stretch modes. As with the studies described above, within seconds of adsorption, the VSF data were invariant with time. Very different dynamics for these two isomers were observed in the interfacial tension data. The atactic PMA showed a large reduction of interfacial tension that continued to drop over 90 min, whereas the isotactic PMA showed a drop in interfacial tension of only a few mN/m. The difference in the dynamic interfacial tension data was attributed to the two different bulk conformations of the isomers. Under our experimental conditions, atactic PMA was in a compact coil configuration, while isotactic PMA was in a more extended configuration. In this case, the driving force for the continued adsorption of atactic PMA to the oil–water interface was attributed to the favored hydrophobic interactions between the coiled polymer and the initial adsorbed layer, whereas continued adsorption of the more extended isotactic PMA to the oil–water interface was unfavorable due to steric hindrance. PAA, similar to atactic PMA, also continues to adsorb to the oil–water interface due to favorable hydrophobic interactions between the coiled polymer and the initial adsorbed polymer layer.

Effects of Ions on Adsorption. The previous studies clearly showed interfacial adsorption of PAA under low pH conditions where the polymer has a low electrostatic charge, and strong desorption above a critical pH where carboxylic acid deprotonation becomes a factor.⁴² Given the charged nature of metal ions in aqueous solution and the propensity of carboxylate ions to chelate to some metal ions, the outstanding question is how the presence of metal ions alters the adsorption of the polymer and the critical pH of desorption found for the metal-free solutions. Beyond intellectual curiosity, the binding of metal ions to a macromolecular assembly is of critical importance to the understanding of protein structure and folding as well as the fate and transport of humic acid–metal complexes, where divalent metal ions play an important role by binding to carboxyl functional groups. To this end, the experiments described herein have been conducted in the presence of both mono- and divalent metal ions.

For PAA in bulk aqueous solution, previous studies showed that carboxylated poly acids bind more strongly to Ca^{2+} than Mg^{2+} , whereas sulfonated poly acids show no difference in the binding behavior between the two divalent ions.^{54,55} To establish a baseline for the metal ion addition studies, VSF spectra of the carbonyl mode of 450 kD PAA (5 ppm) were obtained at two pH values, pH 2 and pH 4. The two signals were identical; thus only the pH 2 fit is shown in Figure 4.

The results in Figure 4 for pH 2 and pH 4 are unambiguous: below the critical pH, the addition of Ca^{2+} or Mg^{2+} has no effect on the population and/or orientation of the polymer at the interface, as seen by the constancy in the carbonyl stretch mode from the carboxylic acid groups on PAA. The results are

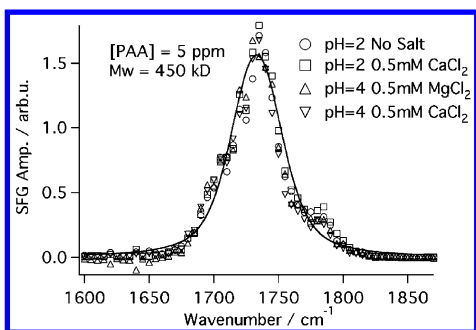


Figure 4. VSF spectra (ssp polarization) of PAA (5 ppm, 450 kD) with Mg^{2+} and Ca^{2+} at the CCl_4 -water interface at pH in the range of 2–4. No increase in resonant amplitude from the carbonyl mode is observed when ions are added to the solution.

strikingly different at pH 4.5 where the polymer was previously shown by VSF to be absent from the interface. At this higher pH, when Ca^{2+} or Mg^{2+} ions are added to solution, spectral features appear that indicate that the metal ions drive the polymer to the interface. The surface tension measurements at this higher pH and in the presence of metal ions also indicate polymer adsorption. As a demonstration of this effect, the VSF spectra shown in Figure 5A and C are taken at pH 4.5 in the absence and presence of Mg^{2+} and Ca^{2+} . As shown at pH 4.5 and in the absence of the metal ions, there is no VSF response from either the carbonyl mode of the carboxylic acid at 1732 cm^{-1} or the carboxylate mode near 1400 cm^{-1} . However, with the addition of Mg^{2+} , the VSF signal appears at both 1730 and 1410 cm^{-1} . The 1410 cm^{-1} peak is assigned to the carboxylate symmetric stretch and is comparable to literature assignments of this mode.⁵⁶ In these studies of PAA, this peak has only been observed when metal ions are present in solution. The carbonyl mode of the carboxylic acid is observed at essentially the same

frequency as was observed for this mode at lower pH's. At either concentration, Mg^{2+} ions appear to drive the adsorption and orientation of PAA at the oil-water interface. This is supported by the interfacial tension measurements shown in Figure 5B. When 1 mM MgCl_2 is added to the solution, the interfacial tension falls to an equilibrium value of $\sim 36\text{ mN/m}$ after 2000 s. In the case of Ca^{2+} , the VSF spectra (Figure 5C) show results similar to those of Mg^{2+} , with the exception of an almost 2-fold increase in VSF intensity over that observed for Mg^{2+} . The increased amplitudes for the Ca^{2+} versus Mg^{2+} VSF spectra are observed for both the carbonyl mode and the carboxylate mode.

Both Mg^{2+} and Ca^{2+} give identical adsorption profiles for the interfacial tension measurements, with increased adsorption as a function of time (Figure 5B and D). However, similar to what was observed in the PAA studies in the absence of metal ions, the appearance of VSF signal for both the carbonyl and the carboxylic acid mode occurs within the first minute and does not change beyond that time. This supports an adsorption process similar to the metal-free polymer adsorption behavior. Metal ion binding to the ionized carboxylate groups results in a rapid diffusion and adsorption of a thin layer of oriented polymer to the interface followed by a buildup of disordered polymer. The ordering of the carboxylate-metal moieties of this ordered polymer layer is accompanied by an ordering of the carboxylic acid groups.

Interestingly, the interfacial tension measurements give identical results for Ca^{2+} and Mg^{2+} with a final equilibrium interfacial tension of $\sim 36\text{ mN/m}$. This is in contrast to the very different VSF spectroscopy data that show a large change in spectral amplitude for Ca^{2+} relative to Mg^{2+} . These results indicate that Ca^{2+} induces a much stronger orientation in the carboxylate and carbonyl groups than the Mg^{2+} , but neither does it induce more adsorption to the interface nor does it bind

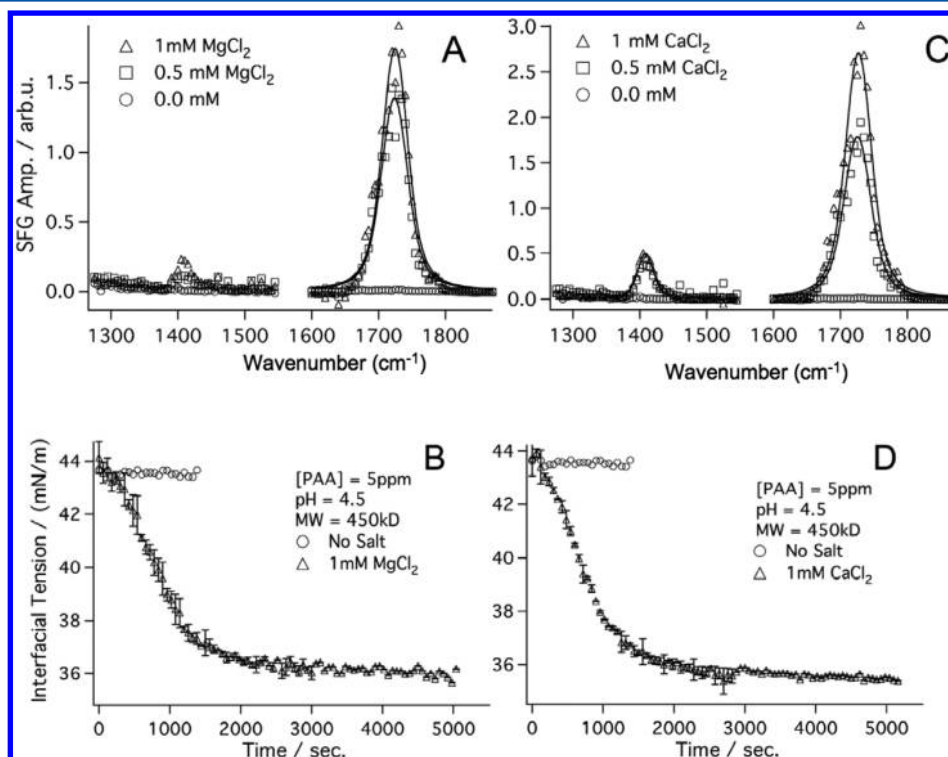


Figure 5. VSF spectroscopy (top, ssp polarization) and interfacial tension measurements (bottom) of PAA with added Mg^{2+} (A,B) and Ca^{2+} (C,D).

more strongly to the PAA carboxylate groups. Molecular orbital studies of calcium and magnesium ion coordination have shown that in hydrated complexes Ca^{2+} has a larger net charge on it than does Mg^{2+} .⁵⁷ This suggests that the field of the calcium ions has a greater ability to screen the carboxylate anionic charge of interfacial PAA, which would in turn produce a stronger orientation of both the carbonyl and the carboxylate groups and result in stronger VSF signal.

Additional evidence of adsorption of PAA to the interface in the presence of ions is found in VSF spectra of the water OH and C–H stretch regions as shown in Figure 6.

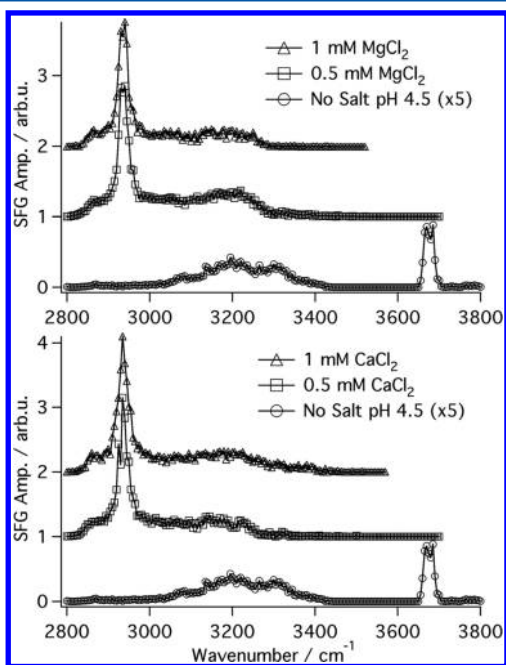


Figure 6. VSF spectra (ssp polarization) of the water and C–H region for Mg^{2+} and Ca^{2+} binding with PAA.

For reference, the PAA VSF spectra at pH 4.5 without ions are also shown in Figure 6. The neat water spectrum obtained is similar to what has been found in previous VSF studies of water at the neat CCl_4 –water interface.^{46,58} Previous studies have shown that this neat CCl_4 –water spectrum is highly sensitive to the presence of trace amounts of impurities, organics, or charged species that migrate to the interface. The absence of any spectral changes in the presence of the polymer in the aqueous phase is a strong indication that no polymer is adsorbed to the interface at pH 4.5. In the presence of ions, however, the water spectra change dramatically, showing intense C–H stretch modes in the 2800–3000 cm^{-1} region and water modes in the 3000–3200 cm^{-1} region, indicative of the strong adsorption and orientation of PAA reflected in the carbonyl region of the VSF spectrum. Like the carbonyl and carboxylate region in Figure 5, the C–H region shows the addition of Ca^{2+} gives more signal than does an equivalent addition of Mg^{2+} , although the difference between the Ca^{2+} and Mg^{2+} C–H intensities is smaller than that between the Ca^{2+} and Mg^{2+} carbonyl and carboxylate intensities. This is likely due to the backbone C–H group orientation having less dependence on the ionic species than the carboxylates, which directly interact with the ions. In addition to the C–H modes, the broad water peak decreases in intensity relative to the C–H modes when going from an ion concentration of 0.5 to 1 mM

for both the Ca^{2+} and the Mg^{2+} . This indicates that increasing the ion concentration leads to fewer oriented water molecules in the interfacial region. We conclude this is due to charge neutralization of the polymer carboxylate anions via the binding of the cations, which acts to decrease the field strength that was a result of the anions at the interface.

Divalent ions generally bind more strongly to PEs than monovalent ions. This is due to the greater ability of the larger divalent ions to penetrate into the cylindrical symmetry of the polymer than smaller monovalent ions.⁵⁹ Na^+ was added to PAA solutions to test whether monovalent ions, like the divalent ions, increase interfacial adsorption for PAA, and to better understand the differences in binding ability of Na^+ compared to Ca^{2+} and Mg^{2+} . The VSF spectra in Figure 7 show

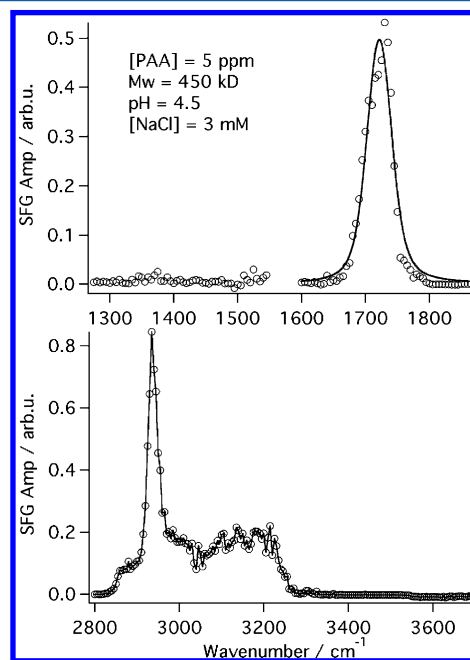


Figure 7. VSF spectra (ssp polarization) of PAA (5 ppm, 450 kD) with NaCl (3 mM) at the CCl_4 –water interface.

that Na^+ does induce adsorption of PAA, which otherwise did not adsorb at the interface at this pH. However, the degrees of adsorption and orientation are lower as seen by the absence of the carboxylate stretch mode in Figure 7 along with the smaller amplitude of the carbonyl stretch mode as compared to what is observed for PAA with Ca^{2+} or Mg^{2+} . The lesser degree of adsorption and ordering is also observed in the smaller C–H stretch mode amplitudes. Nevertheless, the presence of Na^+ ions does cause enough charge neutralization of the PAA carboxylate groups to induce some, albeit lower, degree of ordered assembly at the interface.

Even though it is clear that Na^+ does aid in adsorption through charge neutralization, it is not as effective at screening the charged groups from one another as the divalent cations. This results in the lack of VSF signal observed from the carboxylate functional groups. We can conclude that monovalent ions, at equivalent ionic strengths to divalent ions, do not screen the charge of the carboxylate ions enough to allow orientation of this group at the oil–water interface. Because of the lack of screening provided by the Na^+ , the charged groups must either be adopting opposing orientations along the

backbone of the chain or still be located on the solvated loops that extend into the water phase.

The interfacial tension data shown in Figure 8 support the adsorption model for PAA that has been described earlier in

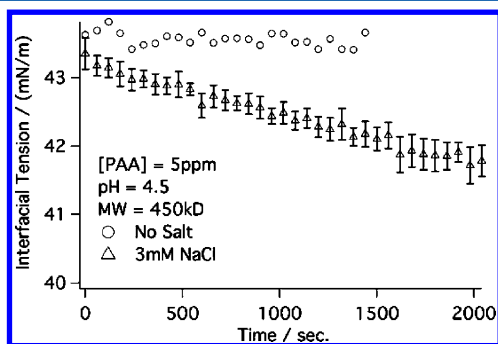


Figure 8. Interfacial tension measurements of PAA (5 ppm, 450 kD) with NaCl (3 mM) at the CCl_4 -water interface.

these studies. As discussed with the previous data, the VSF signal remains invariant with time after a few seconds of adsorption, yet the interfacial tension continues to drop with time. This again provides strong evidence that the adsorption of PAA to the oil-water interface is a multistep process.

The interfacial tension data show, however, that the carboxylate- Na^+ interactions are sufficiently weaker than the carboxylate-divalent cation interactions. As compared to the interfacial tension of PAA without added ions, the addition of NaCl causes the reduction of interfacial tension with time and thus induces the adsorption of PAA to the oil-water interface. This reduction, however, is not as drastic as with the addition of the divalent cations. This indicates that Na^+ neither sufficiently binds to nor adequately screens the carboxylate groups on PAA to cause the same degree of polymer accumulation at the interface over time as Mg^{2+} and Ca^{2+} .

The difference between the degrees of charge screening and interfacial orientation of the PAA carboxylate groups by the Mg^{2+} and Ca^{2+} ions versus the Na^+ ions can be explained by what is known about how counterions of different valencies interact with polyelectrolyte chains in solution. While divalent ions are able to completely penetrate the hydration layer of the PE, which leads to strong interactions between the ions and the charged groups on the polyelectrolyte, the monovalent alkali metals cannot.⁶⁰ This gives the monovalent ions more freedom to travel along the surface of the PE, leading to weaker interactions between the ions and specific charged sites on the PE.

From the data described above, it is clear that the addition of ions increases adsorption of PAA at a solution pH where there would otherwise be no adsorption. A cartoon depicting this increased adsorption at higher pH is shown in Figure 9.

Figure 9 shows that above the critical pH (i.e., pH 4.5), metal ions interact strongly with carboxylate groups both along the backbone of the polymer and through the displacement of solvating water molecules around the carboxylate groups, the net effect being enhanced adsorption of highly oriented polymer at these higher pH's. Because the metal ions screen the anionic charge, the carboxylate functional groups are able to achieve a net orientation at the interface, pointing into the water phase and generating VSF signal. It was clearly observed that Na^+ ions, like the divalent ions, have the ability to enhance PAA adsorption, but lack the capacity to screen the anionic

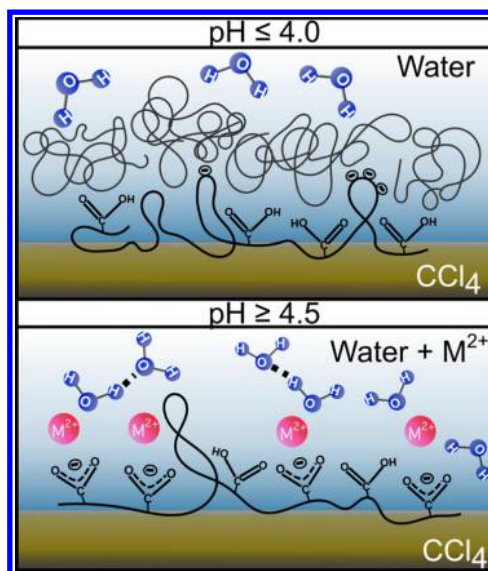


Figure 9. A cartoon representing PAA adsorption at the interface below pH 4.5 (upper panel) and above pH 4.5 (lower panel) in the presence of divalent metal ions.

charge, resulting in a lack of observed carboxylate VSF signal at the interface.

CONCLUSIONS

In summary, we have detailed the first experiments to directly probe the adsorption and orientation characteristics of a polyelectrolyte in the presence of mono- and divalent metal ions at the neat oil-water interface. Vibrational sum frequency spectroscopy was used to monitor the carbonyl, carboxylate, methylene, and water vibrational modes with supplemental data coming from interfacial tension measurements using the pendant drop method. It was found that poly(acrylic acid) adsorbs to the interface under a very specific set of conditions, either below a critical pH or in the presence of ions if above the critical pH.

The implications of this work are 2-fold. First, the fluid nature of interfacial biochemistry necessitates the study of biomolecules in an environment that mimics *in vivo* conditions. The oil-water interface is an excellent medium to probe these important systems, and this work shows how the interface can be used to this degree. While many polyelectrolytes and biomolecules are well characterized in bulk solution, the conformation, adsorption properties, and dynamics can be quite different at the interface as shown in this work. The sharp transition for adsorption versus desorption at a critical pH demonstrates the differences between molecular properties in the bulk phase versus at the interface, particularly in the case where it was expected that neutral polyelectrolyte be in a random coil. This was clearly shown to not be the case at the oil-water interface, indicating that some of the same differences may be observed for biomolecules, particularly in areas where conformational changes may be taking place, such as with protein folding, or enzyme reactions. The second implication of this work is in the area of polyelectrolyte complexation. Many studies of humic acid binding to metal ions are concerned with the fate, transport, and bioavailability of both toxins and nutrients in environmental systems. A deeper understanding of the conformation and interfacial activity of these types of macromolecules in the presence of

ions is of obvious importance with regards to environmental cleanup of toxic metals as well as understanding how nutrients and toxins are placed within the environment. It is clear from the studies presented here that polyelectrolyte adsorption to the oil–water interface can be facilitated by metal binding. Where pH conditions were not initially favorable for PAA adsorption, it was found that very small quantities of ions led to a large increase in surface activity, indicating the potential for carboxylate-containing humic substance to accumulate at fluid environmental interfaces when bound to metal ions and leading to the concentration of both toxins and nutrients in these regions. This work demonstrates an important avenue of investigation for polymers at the oil–water interface and opens the door to studying macromolecules in an environment that is fluid on both sides of the interface, mimicking the true nature of biological and environmental systems.

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Notes

The authors declare no competing financial interest.

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