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The Surface Behavior of Hydrated Guanidinium and Ammonium Ions: A Comparative Study by Photoelectron Spectroscopy and Molecular Dynamics

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Abstract

Through the combination of surface sensitive photoelectron spectroscopy and molecular dynamics simulation, the relative surface propensities of guanidinium and ammonium ions in aqueous solution are characterized. The fact that the N1s binding energies differ between these two species was exploited to monitor their relative surface concentration through their respective photoemission intensities. Aqueous solutions of ammonium and guanidinium chloride, and mixtures of these salts, have been studied in a wide concentration range and it is found that the guanidinium ion has a greater propensity to reside at the aqueous surface than the ammonium ion. A large portion of the relative excess of guanidinium ions in the surface region of the mixed solutions can be explained by replacement of ammonium ions by guanidinium ions in the surface region in combination with a strong salting out effect of guanidinium by ammonium ions at increased concentrations. This interpretation is supported by molecular dynamics simulations, which reproduce the experimental trends very well. The simulations suggest that the relatively higher surface propensity of guanidinium compared to ammonium ions is due to the ease of dehydration of the faces of the almost planar guanidinium ion, which allows it to approach the water-vapor interface oriented parallel to it.

Keywords

XPS, MD simulations, aqueous solutions, air/water interface, liquid micro-jet.

Introduction

The side chains of the two genetically encoded amino acids arginine and lysine contain guanidinium and ammonium groups, respectively, which are positively charged at physiological pH. An improved understanding of the behavior of the guanidinium and ammonium ions may translate into a better characterization of the roles of arginine and lysine in proteins. Arginine and lysine often have similar functions within proteins and peptides, but are not mutually interchangeable. For example, replacing all but the five lysine residues that are functionally essential in a green fluorescent protein with arginine residues enhances the stability of the native structure against chemical denaturation.¹ Replacing lysine residues with arginine residues in cell-penetrating peptides enhances the ability to penetrate cell membranes.² In addition, guanidinium ions ($[\text{C}(\text{NH}_2)_3]^+$, denoted as Gdm^+) and ammonium ions (NH_4^+) themselves are important in biochemistry. Guanidinium salts are commonly used as protein denaturing agents;³ ammonium sulfate is commonly used as a protein crystallizing agent (e.g., in preparation for X-ray or neutron crystallography experiments).⁴

Within the Hofmeister series, which orders ions according to their ability to salt out proteins,^{5,6} which is in turn related to the surface propensity of the ions, the ammonium ion lies close to the center, somewhat on the side of stabilizing ions.⁷ Depending on the molecular context, the hydration properties of ammonium can vary somewhat, but in general it behaves similar to larger alkali cations.⁸ In contrast, the guanidinium functionality can be found on either of the extreme sides of the Hofmeister series depending on the particular process under investigation. Guanidinium mostly behaves as a salting in and destabilizing agent, but can in some cases salt out compounds due to its amphiphilic nature.^{9,10}

Comparing the enthalpies of hydration of the ammonium and guanidinium ions, -329 and -602 kJ/mol,^{11,12} it is found that the guanidinium ion is more strongly hydrated than the ammonium ion overall, but the ammonium ion is more strongly hydrated than the individual amine groups in the guanidinium ion, ca. -200 kJ/mol. However, the hydrogen bonding is stronger, more energy rich, to the guanidinium ion as there are only two hydrogens per

nitrogen participating in hydrogen bonding in each amine group compared to four for the ammonium ion. Furthermore, analysis of surface tension data suggests that ammonium is strongly depleted, though less than the alkali cations, at the water-vapor interface, while the guanidinium ion is predicted to be only weakly depleted.^{11,13–15}

The guanidinium ion, which is composed of a carbon atom surrounded by three amino groups, is planar in solution,¹⁶ due to its quasi-aromaticity,¹⁷ and rotation around the C-N bonds is therefore restricted. This has the consequence that the guanidinium ion can donate hydrogen bonds parallel to its molecular plane only.^{18,19} The flat faces of the ion have a low charge density and the already weak interactions between water and the quasi-aromatic π -system of guanidinium ions is further weakened by the positive charge of guanidinium.¹⁷ The strength of this hydration asymmetry is, however, still under debate (see refs 10 and 20 and references therein). Furthermore, fs-IR pump-probe and neutron scattering experiments suggest that guanidinium ions on average interact with water about as strongly as water molecules interact with each other.^{18,21}

Molecular dynamics (MD) simulations of guanidinium ions in water show an orientation dependent propensity to reside at the water surface.²² While there is a net depletion of guanidinium in the surface region, the simulations predict that a non-negligible population of guanidinium ions is present in the water surface layer. The guanidinium ions residing at the surface are preferentially oriented parallel to it. These ions donate a slightly larger number of hydrogen bonds to water than guanidinium ions in the bulk solution, but their faces toward the vapor side of the interface are almost completely dehydrated.^{22,23} The adsorption of parallelly oriented guanidinium ions at the water surface is qualitatively similar, but much weaker than the adsorption of aromatic compounds, such as benzoic acid, that adsorb strongly with the aromatic rings oriented almost parallel to the water surface.²⁴ MD simulations of ammonium chloride solutions, on the other hand, show a simple surface behavior in that the ammonium ion concentration profile monotonically decay to zero close to the water-vapor interface.²⁵

X-ray Photoelectron Spectroscopy (XPS) on a liquid micro-jet is a surface sensitive technique that has previously been applied to study the chemical composition of surfaces of aqueous solutions, see e.g., refs 26–29. This method has a unique combination of features being both element specific and surface sensitive. In many cases, chemical shifts even allow atoms of the same element in different chemical environments to be distinguished from each other. Here, the fact that both guanidinium and ammonium ions contain nitrogen atoms with distinctly different binding energies in the photoemission (PE) spectrum, was exploited to study their relative surface propensity. Aqueous solutions containing guanidinium and ammonium chloride, and mixtures of these salts, which contain two or three different ionic species, respectively, one of which is always chloride, have been investigated. The ratio of the guanidinium and ammonium N1s PE intensities for mixed solutions were determined for different concentrations. In addition experiments were performed, where the stability of the experimental setup is carefully maintained to allow comparison of recorded PE intensities of different sample solutions. This enabled determination of the change in Cl2p and N1s PE intensities as ammonium ions are gradually replaced by guanidinium ions in solutions with equal ionic strength. The experimental results are compared to MD simulations of the analogous systems in a slab geometry.

Methods

Experimental Details

The XPS measurements were performed at the soft X-ray beamline I411^{30,31} at the MAX IV Laboratory, the Swedish national synchrotron radiation facility, Lund University, Sweden. The experimental setup has been described in detail previously.³² For clarity, an overview of the experimental setup is given here. The sample solution is pushed into the experimental chamber, which is kept under vacuum, through a glass nozzle with an inner diameter of 20 μm . The synchrotron radiation intersects the liquid micro-jet perpendicular to both the

flow direction of the jet and the central axis of a hemispherical electron analyzer, which is mounted at 54.7° (the so called 'magic angle'³³) relative to the polarization plane of the synchrotron radiation. This orientation minimizes angular distribution effects in the resulting photoelectron spectra.³⁴ The emitted photoelectrons enter the electron analyzer through a skimmer, situated approximately 2 mm from the liquid surface and the kinetic energy of the photoelectrons is recorded. The measurements were performed at a distance of 2-3 mm downstream from the nozzle, well before the liquid micro-jet breaks into a train of droplets.³⁵ The sample that has passed the interaction region is collected in a liquid nitrogen-cooled trap.

Aqueous solutions of guanidinium chloride (GdmCl), sodium chloride (NaCl) and ammonium chloride (NH₄Cl) were prepared freshly from commercially available chemicals (Sigma Aldrich) and demineralized water (18.2 M Ω , Millipore Direct-Q). Furthermore, prior to the XPS experiments, each sample was filtered (Whatman Puradisc FP30 syringe filters, 1.2 μm) to remove solid particles, and sonicated to remove air bubbles, both which may disturb the flow in the liquid micro-jet or cause the injection system to fail. Concentrations of the solutions used in the XPS experiments are given in mol $\cdot\text{dm}^{-3}$ (denoted as M).

In all experiments, the relative surface affinity of guanidinium and ammonium ions was monitored via their N1s PE line at 500 eV photon energy, at which also the valence PE spectra of liquid water was recorded. The latter is a measure of the overlap of the X-ray beam with the liquid micro-jet and the entrance of the electron analyzer. The same spectrum is also used to calibrate the energy scale, by aligning the 1b₁ PE line of liquid water to 11.16 eV.³⁶ The Cl2p PE lines were recorded at 300 eV photon energy. These photon energies result in a photoelectron kinetic energy of 90-95 eV. This kinetic energy region is, according to the so-called universal curve,^{37,38} close to that corresponding to the minimum electron attenuation length (EAL), which makes the measurements highly surface sensitive. To the best of our knowledge, an EAL of 5-12 Å for a curved surface is physically reasonable.^{37,38} The PE signal obtained from a distance x to the surface is damped by a factor $\exp(-x/\text{EAL})$,

yielding, e.g., $(1-1/e) \approx 63\%$ of the recorded signal originates from a layer with thickness equal to the EAL.³⁴

In order to compare PE peak areas of different solutions to each other, the spectra must be normalized in a way that accounts for the flux of the X-ray beam. A comparison of recorded PE intensities from different sample solutions is only possible when the spectra are recorded under stable conditions of the experimental setup and the light source. The intensity of the light source and the alignment of the experimental setup can change over time, which results in an increasing or decreasing overall PE intensity. To ensure that these experimental conditions are unchanged over a longer time period, the valence spectrum of a 50 mM LiBr solution, which is used to flush the experimental setup between each sample solution was measured, and the recorded $1b_1$ PE intensities are compared.

For the first data-set, binary mixed solutions of GdmCl and NH_4Cl with equal concentrations, 0.5, 1, and 2 M of each compound, were prepared. The N1s spectra were recorded at different occasions, hence direct comparison between these sample solutions are impractical and only ratios of the two N1s lines within each spectrum will be discussed. For the second data-set, the N1s and Cl2p PE spectra of the pure 2 M GdmCl and 2 M NH_4Cl as well as the mixed solutions containing 2 M GdmCl and either 2 M NaCl or 2 M NH_4Cl and a mixture of 1 M GdmCl and 1 M NH_4Cl were measured. The recorded PE intensities from these experiments were only compared when the PE intensities of the water $1b_1$ signal of the cleaning solution before and after the sample solutions varied by less than 5%.

The curve fitting was done using the SPANCF³⁹ fitting routine for IGOR Pro (WaveMetrics, Inc, Lake Oswego, OR, USA). The N1s peak of guanidinium shows a small asymmetry towards higher binding energies, which probably results from vibrations within the molecule and variations in binding energy with fluctuations in the hydration shell.⁴⁰ PCI profiles (post-collision interaction),⁴¹ were used to fit the N1s PE line of guanidinium. This form was chosen to account for the asymmetry, without any assumptions about the physical mechanism. The N1s PE line of ammonium on the other hand is symmetric and was fitted using

Voigt line shapes. We do not aim for a full analysis of the spectral features; instead the fits are performed on a "best fit" basis. Such a simple approach is possible to apply since only the peak areas are used in the final analysis. For the fitting, the spectra of the pure solutions were used to extract the line shape parameters that were later used to constrain the number of free fitting parameters for the spectra of the mixed solutions. This ensured the use of consistent line profiles throughout the fit, while intensities were free to vary. The energy shift between the two N1s PE lines, arising from the two chemically inequivalent nitrogen atoms, was a free parameter, but constrained to be the same in all spectra of mixed solutions. The resulting energy positions of the individual peaks were consistent (within ± 50 meV) with the values obtained from fits of the spectra from solutions containing only one compound. The PE lines of the Cl2p spin-orbit components were fitted using two Voigt line shapes, where the total area of the Cl2p_{1/2} component was constrained to be half of the area of the Cl2p_{3/2} component, accounting for the statistical weight of the spin-orbit components. This resulted in a robust fit, from which the resulting ratios of the observed PE intensities for the comparison of experimental data to simulated concentration profiles were derived.

In this experiment, the ratios of observed PE intensities are a measure of the relative abundance of two compounds in the surface region. For the direct comparison of N1s PE lines originating from different chemical surroundings, it is assumed that the photoionization cross section in the kinetic energy range used is constant. Even though there is little known about the variation of photoionization cross section of N1s of different ions in aqueous solution, this assumption is reasonable since the kinetic energy region of 90-95 eV is well above possible shape resonances.⁴² Also the possible influence of scattering of the outgoing photoelectron on neighboring atoms,^{43,44} which might modulate the photoionization cross section, is considered to be negligible for the studied systems.

Computational Details

MD simulations of the interface of aqueous guanidinium and ammonium chloride solutions were performed for a set of compositions corresponding approximately to the experimental conditions. We employed the slab geometry; periodic boundary conditions were applied in all directions with some space left between the periodic images of the liquid phase in z -direction. This created an infinite array of alternating vapor and liquid phases, each connected to its periodic replicas in the x and y -directions, but disconnected in z -direction. The dimensions of the simulation box were $43 \times 43 \times 150$ Å. The liquid phase was roughly 40 Å thick, depending slightly on system composition.

The cation compositions for individual simulation runs are shown in Table 1. The number of water molecules was always 1977 and the number of chloride counter ions was equal to the total number of cations. The systems in run 1, 2, and 4 correspond approximately to 2 M solutions, while the ionic strength is doubled in run 5 and halved in run 3. We used the SPC/E water model.⁴⁵ The force field parameters for chloride and ammonium ions used were taken from refs 46 and 47, respectively. The force field for the guanidinium ion is based on, but not identical to, the force field reported in ref 22, which uses geometric combination rules for the Lennard-Jones σ parameters. To ensure that the guanidinium force field was compatible with the Lorentz-Berthelot combination rules, used for ammonium and chloride, Lennard-Jones parameters were selected to reproduce the bulk radial distribution functions of the force field reported in ref 22. We did not use a polarizable force field as it is not expected that polarization of the cations in this study is decisive for their surface propensity (see SI of ref 22), in contrast to the situation for, e.g., heavier halides.⁴⁸

Initial coordinates were generated starting from a previously equilibrated guanidinium chloride slab at high concentration, by randomly deleting and/or replacing ions to give the correct composition. The original slabs were created from bulk simulations by extending the simulation box length in z -direction. The systems were equilibrated for 0.5 ns and the length of all production runs was 100 ns. In all cases the time step was 1 fs. The dimensions

of the system were held constant and temperature was kept at 300 K using the Berendsen thermostat.⁴⁹ The cut-off for short-range interactions was 9 Å, and long-range electrostatics were handled using the particle-mesh Ewald method with a grid spacing of approximately 1 Å.⁵⁰ Simulations were performed using the AMBER11 program package.⁵¹

Table 1: Number of cations of each species in each simulation run

run	Gdm ⁺	NH ₄ ⁺
1	72	0
2	0	72
3	18	18
4	36	36
5	72	72

A simple attenuation model was employed to calculate the expected PE intensities from the simulated concentration profiles. Under the assumption that the PE signal of a species i , I_i , is proportional to³⁸

$$I_i \propto \int_{z_0}^{\infty} c_i g_i(z) \exp\left(-\frac{1}{\lambda} \int_{z_0}^z g^{tot}(z') dz'\right) dz, \quad (1)$$

ratios of PE intensities can be computed from the concentration profiles. The distribution function $g_i(z)$ is the concentration profile of species i normalized by its bulk concentration. $g^{tot}(z)$ is the distribution function corresponding to the total concentration of all species and c_i is the actual concentration of component i . The z -coordinate is perpendicular to the surface and with z_0 located in the vapor phase, where all density functions have dropped to zero, the integral runs towards the water bulk. For molecular species containing only one heavy atom, its position is used to compute the concentration profile. For guanidinium, the position of the central carbon atom is used. The concentration in the middle of the slab was used as a proxy for the bulk concentration for the purpose of computing $g_i(z)$. This concentration deviated from the nominal concentration by up to 20% due to the partitioning of ions between the surface and the slab interior. To partially compensate for this deviation, the nominal concentration was used for c_i in eq 1. The remaining error depends on the rate of variation

of $g_i(z)$ with concentration and is discussed in the supplementary information (SI). λ is the effective attenuation length (EAL), which is in the order of molecular dimensions.^{37,38} The integral in the exponent may be thought of as a density-weighted z -coordinate, i.e., a measure of the amount of material that emitted electrons have to penetrate. The concentration profiles for the two slab interfaces were averaged. Beyond the range where the concentration profiles could be obtained from simulation, corresponding to half the slab thickness, their normalized value was assumed to be unity, corresponding to bulk concentration.

Results and Discussion

Relative surface propensities of GdmCl and NH₄Cl in equimolar mixed solutions

The N1s PE intensities of guanidinium and ammonium ions were measured in mixed aqueous solutions containing 0.5, 1, and 2 M each of GdmCl and NH₄Cl. Pure solutions of 2 M GdmCl and 2 M NH₄Cl, respectively, were used to obtain line shapes to constrain fitting parameters. The N1s binding energy of the three equivalent nitrogen atoms in the guanidinium ion was determined to be 405.17 eV whereas the corresponding binding energy of the nitrogen in the ammonium ion was found at about 1.57 eV higher energy, i.e. 406.74 eV, see Figure 1. Since the binding energies cannot be determined with an accuracy better than ± 50 meV in this experiment, the N1s spectra of a given species were shifted within these boundaries to align their peak positions to the same binding energy. The full linewidths at half maximum are 1.26 and 1.56 eV, respectively. Despite these widths, the difference in peak positions for guanidinium vs. ammonium is sufficient to accurately resolve the peaks in the spectra of solutions containing both ions.

The N1s spectra of the three equimolar, mixed guanidinium and ammonium chloride solutions are shown in Figure 1, where the intensity scales of the different traces differ from each other. For visual clarity, we chose to normalize the spectra of the mixed solutions to

the N1s PE intensity of ammonium. Note that the guanidinium ion contains three nitrogen atoms, whereas the ammonium ion contains only one; a relative peak area of 3:1 would correspond to equal surface propensities, assuming that the N1s photoionization cross section is equal for both species. However, for the 0.5 M mixed solution, the guanidinium N1s PE intensity is more than four times higher than the ammonium N1s PE intensity. Furthermore, the N1s PE intensity of the guanidinium ion increases more strongly with increasing concentration than the N1s PE intensity of the ammonium ion, see Figure 1, which will be discussed in detail later.

Figure 1: N1s photoelectron spectra of the equimolar mixed and pure GdmCl and NH_4Cl solutions, obtained at 500 eV photon energy. Experimental data are shown as points, the dashed lines are the individual components and the solid lines represent the resulting total fit curves. For easy comparison, the spectra of the mixed solutions are normalized to the N1s intensity of ammonium.

The simulated concentration profiles of the corresponding systems are shown in the left panel of Figure 2. For clarity the curves have been offset and the vertical lines indicate where the concentration is half the bulk value as guidance for the eye. In each of the guanidinium ion profiles, there is a peak/shoulder located approximately $3 \text{ \AA}$ below the surface. This feature is due to guanidinium ions oriented parallel to the surface, which can approach the

surface more closely than guanidinium ions in other orientations, see Figure 2 in ref 22. For higher concentrations this feature gets more pronounced, which is reflected in the increasing PE intensity of Gdm^+ obtained from XPS measurements. The normalized ammonium concentration profiles are almost independent of system composition, confirming that the ammonium ion has a simple surface behavior with monotonously decreasing interfacial concentration upon approaching the surface (for comparison to related XPS measurements, see SI). For chloride, the shape of the concentration profiles is similar between various concentrations, but the profiles are slightly shifted towards the interface and show a more rapid decline to zero for higher concentrations.

In the right panel of Figure 2 the experimentally observed N1s PE intensity ratios, defined as $R = \frac{I(\text{Gdm}^+)}{(3 \cdot I(\text{NH}_4^+))}$, where the factor three in the denominator accounts for the fact that there are three nitrogen atoms per guanidinium ion, are displayed as a function of total concentration together with the ratios obtained from the simulated concentration profiles using eq 1 for different EALs. The bulk proportions are indicated by a dashed line. Qualitatively, the MD simulations are in good agreement with the results from the XPS experiments. EALs in the range of 3-5 Å reproduce the experimental values. The resulting ratios are larger than unity and increase sub-linearly with increasing concentration. This indicates that guanidinium ions have a stronger surface propensity compared to ammonium ions. Note, however, that the simulations as well as literature surface tension data,¹¹ show that guanidinium ions are depleted at the surface compared to bulk; 'stronger' should thus be interpreted as 'less negative'.

Figure 2: Left panel: Simulated concentration profiles, normalized by the concentration in the middle of the slab, from which the simulated signal ratios are calculated. The curves have been offset for clarity and vertical lines show where the concentration is half the bulk value. Right panel: $I(\text{Gdm}^+)/ (3 \cdot I(\text{NH}_4^+))$ signal ratios as function of the total concentration in equimolar solutions of GdmCl with NH₄Cl, are displayed together with simulated values for a range of electron attenuation length of 3-5 Å.

PE intensity comparison between mixed GdmCl solutions

The results from mixed solutions presented in the previous section show that the guanidinium ion has a higher surface propensity than the ammonium ion. However, the study of the mixed solutions alone cannot determine to what extent this is due to an intrinsically higher surface propensity of guanidinium and to what extent the difference in surface propensity is caused by interactions between the different ions. Scenarios where ammonium “salts out” guanidinium to the surface or guanidinium “salts in” ammonium from the surface, cannot be ruled out at this stage. In order to address this issue, we performed experiments where recorded N1s and Cl2p PE intensities between aqueous solutions of pure 2 M GdmCl, pure 2 M NH₄Cl, mixed solutions containing 2 M GdmCl and either 2 M NaCl or 2 M NH₄Cl and a mixture of 1 M GdmCl with 1 M NH₄Cl are compared directly. Normalized to their bulk concentration, ratios of the observed PE intensities from guanidinium, ammonium, and

chloride ions from mixed solutions are computed by comparing their PE intensity to the respective intensity obtained from the pure GdmCl solution and the pure NH_4Cl solution. The resulting ratios are shown together in Figure 3. Note, as we are computing ratios of the same element in the same species in different solutions, no assumptions for the photoionization cross section is underlying for this part of the analysis.

Figure 3: PE intensity ratios of the aqueous solutions of pure 2 M GdmCl, pure 2 M NH_4Cl , mixed solutions containing 2 M GdmCl and either 2 M NaCl or 2 M NH_4Cl and a mixture of 1 M GdmCl with 1 M NH_4Cl . The corresponding PE spectra are shown in Figure 4 and 5.

To investigate how ammonium ions affect the surface propensity of guanidinium ions, the N1s PE intensity of the 2 M pure GdmCl solution is compared to the N1s PE intensities of mixed solutions containing additionally either 2 M NH_4Cl or 2 M NaCl, see Figures 3 and 4, where an increase in PE signal of the guanidinium ion in the presence of both NaCl and NH_4Cl can be seen. The observed N1s PE intensity of Gdm^+ increases with about 15% for the solution containing 2 M GdmCl and 2 M NH_4Cl compared to the pure 2 M GdmCl solution. An even more pronounced increase (more than 30%) is observed for the solution containing 2 M GdmCl and 2 M NaCl.

If instead the N1s PE line of ammonium ions are compared between the pure 2 M NH_4Cl solution and the mixed 2 M GdmCl with 2 M NH_4Cl solution, see Figures 3 and 4, it can be

seen that it is decreased by about 20% for the mixed solution. This analysis of the N1s PE lines suggests that in these cases the guanidinium ion is salted out to the surface by a second salt present in these concentrated solutions. Ammonium ions, on the other hand, are salted in. Note that the mixture with ammonium ions shows a less strong salting out effect than the mixture with the strongly hydrated sodium ions. Thus, the results from XPS experiments suggest that the net effect is a partial replacement of ammonium ions by guanidinium ions at the surface of the mixed solutions.

Figure 4: The N1s photoelectron spectra of aqueous solutions of pure 2 M GdmCl, pure 2 M NH₄Cl, mixed solutions containing 2 M GdmCl and either 2 M NaCl or 2 M NH₄Cl and a mixture of 1 M GdmCl with 1 M NH₄Cl obtained at 500 eV photon energy. Experimental data are shown as points, the dashed lines are the individual components and the solid lines represent the resulting total fit curves. Note that the relative PE intensity scale is the same for all traces. The measured Gdm⁺ PE intensity increases by adding either NaCl or NH₄Cl and the NH₄⁺ PE intensity decreases when GdmCl is added to the solution. The corresponding computed PE ratios are given in Figure 3.

The total excess or deficit of cations in the surface region must be matched by a corresponding excess or deficit of anions due to the fact that the surface region is overall electrically neutral. The Cl2p PE intensities can thus indirectly be used as a measure of the total amount of cations in the surface region. For this purpose, the measured Cl2p PE intensities were compared between pure solutions of 2 M GdmCl and 2 M NH₄Cl and the mixed solution containing both 1 M GdmCl and 1 M NH₄Cl. Note that all three solutions have the same

concentration of Cl^- ions. The Cl2p PE spectra for the three solutions are shown in Figure 5. The observed PE intensity is significantly lower for the pure 2 M NH_4Cl solution compared to either of the solutions that contain guanidinium ions.

Figure 5: The Cl2p photoelectron spectra of aqueous solutions of 2 M GdmCl, 1 M GdmCl + 1 M NH_4Cl , and 2 M NH_4Cl recorded at 300 eV photon energy. Experimental data are shown as points, the dashed lines are the individual components and the solid lines represent the resulting total fit curves. Cl2p PE intensities for Gdm^+ containing solutions is higher compared to the pure NH_4Cl solution. The computed Cl2p PE ratios are given in Figure 3.

The Cl2p PE intensities relative to the intensity from the 2 M NH_4Cl solution are shown in the right panel of Figure 6 together with the corresponding PE ratios calculated from eq 1. The corresponding simulated concentration profiles are shown in the left panel of Figure 6. The chloride concentration profiles obtained from the MD simulation have the same shape for all three solutions, but are closer to the surface in the Gdm^+ -containing solutions. The measured Cl2p PE intensities from the two Gdm^+ -containing solutions are mutually indistinguishable considering the experimental error bars, and both correspond to an approximately 20 % increase in Cl2p signal compared to the pure NH_4Cl solution. The simulations show a sub-linear increase in the observed PE intensity ratio, which, for an EAL range of 3-5 Å, is consistent with the experimental results. Thus, this analysis supports the previous finding that guanidinium has a higher surface propensity than ammonium ions.

By detailed inspection of the simulated concentration profiles of the guanidinium contain-

ing slabs, shown in the left panel of Figure 6, it can be seen that the Gdm^+ peak/shoulder is more pronounced in the mixed solutions at the same total concentration compared to the pure GdmCl solution. The curves have been offset for clarity and the vertical lines for each component show where the concentration is half the bulk value. Comparing the equivalent experimentally obtained Gdm^+ N1s PE intensities from the 1 M GdmCl + 1 M NH_4Cl solution and the pure 2 M GdmCl solution per guanidinium ion, an increase of about 15% is found, see Figure 3. From both experimental and computational results it can be concluded that an equally increased Cl^- signal for both guanidinium containing solutions in combination with a significantly increased Gdm^+ signal for the mixed solution compared to the pure GdmCl solution, see Figure 3, can be attributed to an increased concentration of guanidinium ions at the surface. A mere replacement of ammonium by guanidinium ions at the surface cannot explain the increased Cl2p PE signal, which instead presumably originates from salting out of guanidinium ions by ammonium ions.

Figure 6: Left Panel: Simulated concentration profiles, normalized by the concentration in the middle of the slab, from which the simulated signal ratios are calculated. The curves have been offset for clarity. Right panel: Cl2p PE ratios relative to Cl2p from the pure 2 M NH_4Cl solution (see Figure 3) as a function of Gdm^+ concentration for a 2 M total salt concentration are shown together with simulated PE ratios for a range of EALs.

Similar effects have been observed in mixed solutions of NaCl and NaBr, where the larger and more polarizable bromide ion was found to be salted out by sodium chloride.²⁸ In the same work, it was concluded that the ion which is less depleted from the surface can realize the gain in free energy associated with replacing, to some extent, the stronger depleted ion at the surface, without simultaneously paying the free energy cost of increasing the surface charge density.

Short-range repulsive ion-ion interactions keep the hydrated ions apart, which can lead to segregation of the species in the surface region in order to lower the systems total energy. Due to their smaller size and therefore larger charge density, ammonium ions are more strongly hydrated than guanidinium ions. The latter are overall more weakly hydrated, due to their lower charge density and weakly hydrated faces (fewer sites for hydrogen bonding), which may be partially dehydrated if the ion is aligned parallel to the water-vapor interface,²² as described in the Introduction. Striving for full hydration, at higher concentrations, when the amount of water molecules per ion available is reduced, a competition between the cations to fill their hydration shells takes place. It is then energetically most favorable if the more strongly hydrated species is fully hydrated in the bulk, and the less strongly hydrated species populates the less favorable surface sites, where complete hydration is impossible. All in all, this leads to an enhancement of guanidinium ions at the aqueous surface, which becomes even stronger when the number of water molecules per ion available to form a hydration shell decreases at higher concentrations.

Though a quantitative comparison between measured PE intensities ratios and ratios obtained from MD simulated concentration profiles is confounded by considerable uncertainty in the electrons EAL, values in the range 3-5 Å reproduce the experimental values with only minor deviations in each of the comparisons above. These EALs are slightly below the lower bound of the range of EALs previously estimated for XPS on a liquid micro-jet,^{37,38} which may indicate that the simulations underestimate the surface propensity of guanidinium ions. Also, a partial dehydration of guanidinium ions at the aqueous surface might be stronger

than for typical cations, based on previous simulations reported in ref 22, which would yield a stronger PE signal. Nevertheless, the simulations appear to give a qualitatively satisfactory picture of the experimentally observed trend with varying concentration. A similar conclusion was drawn in another work on benzoic acid and benzoate in aqueous solutions where photoemission studies were complemented by MD simulations.²⁴

Conclusions

The relative surface propensity of ammonium and guanidinium ions in pure and mixed solutions was studied by photoemission experiments, which were complemented by molecular dynamics simulations. In mixed solutions, the population of guanidinium ions at the surface was found to be significantly enhanced, and the strength of this effect correlates positively with increasing concentration. Measurements were performed under sufficiently stable conditions to allow comparison between photoelectron intensities from different solutions, in addition to different species within the same solution. This allowed the mutual effect of the salts on their respective surface propensity to be disentangled from the intrinsic difference in surface propensity. The direct monitoring of ammonium and guanidinium ions via their N1s PE lines was supplemented by monitoring the variation in the anions abundance through the Cl2p PE intensity. In line with the results from MD simulations, it was found that guanidinium ions have a stronger surface propensity than ammonium ions. A large portion of the relative excess of guanidinium ions in the surface region of the mixed solutions could be explained by replacement of ammonium ions by guanidinium ions in this region in combination with a salting out effect of guanidinium by ammonium ions at increased concentrations. The greater surface propensity of guanidinium ions originates to a large extent from the ease of dehydration of the faces of the almost planar guanidinium ion, which allows the ion to approach the aqueous interface when oriented parallel to it.²²

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Supporting Information Available

Supporting information about the computational and experimental details and results are found in the supporting information: a XPS study of NH₄Cl in aqueous solutions at different concentrations; parameters of the guanidinium model used in MD simulations; error estimation of simulated PE intensity ratio. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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