

# Anomalous Surface Behavior Of Hydrated Guanidinium Ions Due To Ion Paring

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## Abstract

Surface affinity of aqueous guanidinium chloride (GdmCl) is compared to the one of aqueous tetrapropylammonium chloride (TPACl) with addition of sodium chloride

(NaCl) or disodium sulfate ( $\text{Na}_2\text{SO}_4$ ). The experimental results have been acquired using the surface sensitive technique X-ray photoelectron spectroscopy on a liquid jet. Molecular dynamics (MD) simulations have been used to produce radial distribution functions and surface density plots. The surface affinity of  $\text{TPA}^+$  and  $\text{Gdm}^+$  both increases when adding NaCl to the solution. With the addition of  $\text{Na}_2\text{SO}_4$  the surface affinity of  $\text{TPA}^+$  is increased while in the case of  $\text{Gdm}^+$  it decreases. In the MD simulations it is seen that  $\text{Gdm}^+$  and  $\text{SO}_4^{2-}$  form an ion pair which explains the decreased surface affinity of  $\text{Gdm}^+$ , since  $\text{SO}_4^{2-}$  avoids the surface due to the double charge, i.e. the  $\text{Gdm}^+\text{-SO}_4^{2-}$  ion pair will reside deeper than the  $\text{Gdm}^+$  ions. While  $\text{TPA}^+$  does not form ion pairs with  $\text{SO}_4^{2-}$  the  $\text{TPA}^+$  ions are instead enriched at the surface.

## Introduction

How ions interact and affect each other directly and indirectly in aqueous solutions is important for many systems but can be quite complex to understand. Often the surface of the solution is important, and for some systems the surface can have a different composition and concentration compared to the bulk. The surface affinity of solutes can be affected to a large extent by the addition of ions though the mechanism behind can vary. In most cases the surface affinity of solutes increases with addition of ions and have been reported for both inorganic<sup>1</sup> and organic solutes.<sup>2</sup> In both cases with the addition of ions there are not enough water molecules to fully hydrate the solutes and as a consequence the strongest hydrated solutes stay in the bulk while the weaker hydrated solutes reside at the surface. Considering ions, in general the higher charge an ion has the more water is needed in order to screen the charge,<sup>3</sup> i.e. the stronger it is hydrated. This is an important mechanism that is used in the discussion later.

Another example where the addition of salt changes the surface composition but now with a different mechanism, is proteins being denatured by salts. With the denatured protein's new structure its required solvation energy could increase and as a consequence it could

resides at the surface instead, i.e. salting out. Understanding how proteins behave and how to change them are crucial topics in biochemistry, and for some of these applications salting out proteins is an important tool. The strength of an ion's ability to salt out proteins have been ranked by the Hofmeister series.<sup>4-6</sup> But sometimes the real situation does not follow the prediction of the Hofmeister series and is more complex. One such case is the salt guanidinium chloride  $C(NH_2)_3^+Cl^-$  (GdmCl) which acts as a strong denaturant agent while guanidinium sulfate acts as a stabilizing agent.<sup>7</sup> In a combined molecular dynamic (MD) and circular dichroism spectroscopy study<sup>8</sup> the effect of changing the counter ions of tetrapropylammonium  $N((CH_2)_2CH_3)_4^+$  (TPA<sup>+</sup>) from chloride ( $Cl^-$ ) to sulfate ( $SO_4^{2-}$ ) on the conformational stabilities of tryptophan zipper and  $\alpha$ -helical peptides was studied. It was suggested that changes in the conformational stabilities were due to whether or not the sulfate ions were forming an ion pair with Gdm<sup>+</sup> and TPA<sup>+</sup>. Here we investigate if Gdm<sup>+</sup> and TPA<sup>+</sup> form an ion pair with sulfate in an aqueous solution by measuring the surface signal using X-ray photoelectron spectroscopy (XPS) and comparing the results with MD simulations.

The guanidinium cation is not only used in biochemistry but is also a functional group of the amino acid arginine. Peptides rich of arginine have the ability to enter cells at a higher rate than lysine-rich peptides.<sup>9</sup> MD simulations have predicted that Gdm<sup>+</sup> ions can form ion pairs in lipid bilayers which may imply that arginines in such a bilayer can come closer to each other and make the peptide compress and thus gain a lower free energy of penetration<sup>10</sup>. A better understanding of how guanidinium behaves could elucidate the role of arginine in peptides.

# Method

## Computational details

We performed 100 ns molecular dynamics simulations of neat 0.5 m aqueous solutions of guanidinium chloride and tetrapropylammonium chloride as well as mixed solutions of GdmCl and TPACl with additional 1 m Na<sub>2</sub>SO<sub>4</sub> or 1.5 m NaCl at the solution/vapor interfaces. All systems were first equilibrated for at least 1 ns. For solution/vapor interfaces, MD simulations were performed at a constant temperature of 298 K employing the velocity-rescale thermostat.<sup>11</sup> In case of neat solutions, the unit cell contained 18 cations, 18 anions and 2008 SPC/E<sup>12</sup> water molecules. In case of mixed solutions, additional 72 Na<sup>+</sup> and 36 SO<sub>4</sub><sup>2-</sup> ions were added to neat solutions. The size of the unit cell was ca. 4 nm \* 4 nm \* 15 nm, with the thickness of the aqueous phase being ca. 5 nm. Parameters for the ions were taken from.<sup>13,14</sup> Electronic polarization effects were accounted for in an effective way by introducing ECC,<sup>15-17</sup> which is practically realized by rescaling all ionic charges by  $\frac{1}{\sqrt{\epsilon_{el}}}$ , where  $\epsilon_{el} = 1.78$  is the electronic part of the static relative permittivity of water (i. e., the scaling factor equals to 0.75). 3D periodic boundary conditions were used with long range electrostatic interactions beyond the non-bonded interaction cutoff of 0.9 nm being accounted for using the particle-mesh Ewald procedure.<sup>18</sup> The SHAKE algorithm<sup>19</sup> was employed to constrain all bonds containing hydrogen atoms. Equations of motion were integrated using the leap-frog algorithm with a time step of 1 fs. All MD simulations were performed using the program AMBER 11.<sup>20</sup> Radial distribution functions have been rescaled such that they level out to 1 at large distances, which they did not automatically since a bulk method for calculating radial distribution functions have been applied for our systems with surfaces.

## Experimental details

The measured aqueous solutions are listed in Table 1 with details regarding preparation described later. The concentrations of the solutions are such that the compound of interest

(guanidinium and tetrapropylammonium) are the same, 0.5 molal, and the concentration of the additional salts are such that the total concentration of added ions is the same. All measurements were conducted at the undulator beamline I411 at MAXIV, Lund, Sweden;<sup>21,22</sup> combining a liquid jet with the surface and chemically sensitive X-ray Photoelectron Spectroscopy. The technique has been described in detail before.<sup>23</sup> Briefly, the liquid samples are injected into the vacuum system by a HPLC pump through a 20  $\mu\text{m}$  glass nozzle and are after about 1 mm downstream illuminated by the X-rays. Perpendicular to the surface of the liquid jet the entrance of the Scienta SESR4000 analyser was mounted in  $54,7^\circ$  (the so-called magic angle<sup>24</sup>) relative to the polarization plane of the synchrotron light in order to cancel out angular emission effects.<sup>25</sup> The photon energy has been chosen so that the kinetic energy of the electrons is in the most surface sensitive region on the universal curve of mean free path,<sup>26,27</sup> i.e. about 95 eV. The photoelectron signal is then a measure of the abundance of the corresponding ions at the surface. All samples have been prepared with deionized water (18.2  $M\Omega$ , Millipore Direct-Q), ultra sonicated and filtered (Whatman Puradisc Fp30 syringe filter with a pore size of 1.2  $\mu\text{m}$ ). All the spectra have been normalized to the acquisition time and synchrotron ring current, which to the first approximation is proportional to the integrated photon flux. In the cases where comparing the total intensity have been important the samples have been measured in direct succession monitoring stability by running 50mM LiBr solutions before, in between and after each sample and comparing the emission from the 1b1 liquid water valence state. The data have been calibrated to the 11.16 eV 1b1 liquid water valence state<sup>28</sup> and analyzed using IGOR Pro (WaveMetrics, Lake Oswego, OR) with the fitting procedure SPANCF.<sup>29</sup> The curves have been fitted with an asymmetric Voigt profile to accommodate for lifetime, experimental broadening and inelastic contributions. The area of the peaks have been used in the analysis to get the relative difference in affinity of the ions when comparing the samples.

Table 1: The two sets of aqueous solutions

Compound 1		Compound 2
0.5 molal GdmCl		
0.5 molal GdmCl	+	1.0 molal Na <sub>2</sub> SO <sub>4</sub>
0.5 molal GdmCl	+	1.5 molal NaCl
<hr/>		
0.5 molal TPACl		
0.5 molal TPACl	+	1.0 molal Na <sub>2</sub> SO <sub>4</sub>
0.5 molal TPACl	+	1.5 molal NaCl

## Results and discussion

The experimental XPS results for the GdmCl solutions are presented in Fig. 1(a) where the N 1s photoelectron signal for the GdmCl + NaCl solution (blue dotted) is higher than for the GdmCl solution without additional ions (red solid), which in turn has a higher signal than the GdmCl + Na<sub>2</sub>SO<sub>4</sub> (green dashed) solution. Since the bulk concentrations of Gdm<sup>+</sup> ions in the three solutions are the same, the differences in photoelectron signal corresponds to differences in surface concentration of Gdm<sup>+</sup>. This is due to the inelastic mean free path for the electrons which makes the probability for an electron reaching the detector less probable the deeper into the liquid jet the electron originated from. Hence the spectra tells us that compared to the GdmCl solution without additional ions there are more Gdm<sup>+</sup> ions at the surface in the solution with NaCl and less Gdm<sup>+</sup> ions at the surface in the solution with Na<sub>2</sub>SO<sub>4</sub>. The MD simulated density profiles of GdmCl are presented in Fig. 1(b) comparing the Gdm<sup>+</sup> profiles (black) one can see that the surface affinity is smallest for the sample with Na<sub>2</sub>SO<sub>4</sub> added, the largest with NaCl added and in between the sole GdmCl solution - which is in agreement with experimental results. The photoelectron signal is proportional to the amount of substance at the surface but exact surface concentrations are hard to derive and give large uncertainties and we refrain from doing a direct comparison of values to simulation data. What can be compared, and specifically in this case, is the relative change of the Gdm<sup>+</sup> ion surface affinity comparing the solution without additional ions to the solutions with the extra salt added. Making that comparison the experimental and the simulated data shows the same trend, i.e. by adding NaCl to an aqueous GdmCl solution

with the given concentrations the more  $\text{Gdm}^+$  ions resides at the surface, whereas when adding  $\text{Na}_2\text{SO}_4$  to the an aqueous  $\text{GdmCl}$  solution with the given concentrations the  $\text{Gdm}^+$  ions resides at the bulk to a higher degree.

The experimental data from the aqueous  $\text{TPA}^+$  solutions are presented in Fig. 2(a) where the N 1s photoelectron signal for both the shown  $\text{TPACl} + \text{NaCl}$  solution (blue dotted) and  $\text{TPACl} + \text{Na}_2\text{SO}_4$  solution (green dashed) are higher than for the  $\text{TPACl}$  solution (red solid) without additional ions. In other words there are more  $\text{TPA}^+$  ions at the surface in the solutions with the extra salt added compared with the  $\text{TPACl}$  solution without extra salt. Note that there are even more  $\text{TPA}^+$  ions present at the surface in the solution with  $\text{Na}_2\text{SO}_4$  compared with the solution with  $\text{NaCl}$ , which will be discussed later. The MD-simulated  $\text{TPACl}$  density profiles are presented in Fig. 2(b-d), comparing the  $\text{TPA}^+$  profiles (black) one can see that the surface affinity is largest for the sample with  $\text{Na}_2\text{SO}_4$  added, the second largest with  $\text{NaCl}$  added and smallest the neat  $\text{TPACl}$  solution - which is in line with the experimental results. Both  $\text{Gdm}^+$  and  $\text{TPA}^+$  seem to have their surface affinity increased when adding the  $\text{NaCl}$ . As discussed in the introduction, we believe that in the competition of staying hydrated, the  $\text{Gdm}^+$  and  $\text{TPA}^+$  ions are both energetically driven to reside at the surface. What is surprising and interesting is that the  $\text{Gdm}^+$  and  $\text{TPA}^+$  ions in mixture with  $\text{Na}_2\text{SO}_4$  show an opposite behavior, the surface affinity of  $\text{Gdm}^+$  is decreased while increased for  $\text{TPA}^+$ .

The MD simulations offer an explanation for the decreased surface affinity of  $\text{Gdm}^+$ . Just as suggested by Dempsey et al.<sup>8</sup>, the  $\text{Gdm}^+$  ions are in the MD simulations seen to be forming an ion pair with the  $\text{SO}_4^{2-}$  ions. In Figure 3, the radial distribution functions (RDF) for the solutions with the extra added salt are displayed. In blue dots the RDF between the carbon atom in  $\text{Gdm}^+$  and the chloride for the  $\text{GdmCl} + \text{NaCl}$  solution, in green solid line the RDF for the carbon atom in  $\text{Gdm}^+$  and the sulfur atom in sulphate for the  $\text{GdmCl} + \text{Na}_2\text{SO}_4$  solution are depicted. The distinct features at 4 Å for the  $\text{GdmCl} + \text{NaCl}$  solution and the feature at about 4.5 Å for the  $\text{GdmCl} + \text{Na}_2\text{SO}_4$  solution predicts that the

Gdm<sup>+</sup> ions form ion pairs with both Cl<sup>-</sup> in the solution with added NaCl and with SO<sub>4</sub><sup>2-</sup> in the solution with added Na<sub>2</sub>SO<sub>4</sub>. The second feature for the sulfate solution at roughly 7.3 Å is an indication of that there might even be a second Gdm<sup>+</sup> ion attached with the sulfate or that there are two different configurations in which they can form an ion pair. In orange dashed line the RDF between the nitrogen atom in TPA<sup>+</sup> and the chloride ion for the TPACl+NaCl solution, in black dash-dotted the RDF for the nitrogen atom in TPA<sup>+</sup> and the sulfur atom in sulphate for the TPACl+Na<sub>2</sub>SO<sub>4</sub> solution are depicted. Judging by the width and the height of the features at roughly 5 Å for the TPACl+NaCl solution TPA<sup>+</sup> seems to form an ion pair to some degree with chloride, though not as distinct as for Gdm<sup>+</sup> and with a larger distance. With the lack of distinct peaks the TPA<sup>+</sup> does not seem to form an ion pair with sulphate, though there is a feature at roughly 5 Å which corresponds to an ion pair the peak is less intense and broader than the corresponding one for Gdm<sup>+</sup>. Also the peak height of the feature at 5.0 Å is lower than the far end at 10 Å, indicating that, on average, the TPA<sup>+</sup> and the sulphate stay apart.

The SO<sub>4</sub><sup>2-</sup> ions are doubly charged and find it favorable to avoid the surface, and apparently even when forming an ion pair with Gdm<sup>+</sup> the pair will reside deeper than the Gdm<sup>+</sup> ions in pair with Cl<sup>-</sup>. If the ion pairing with SO<sub>4</sub><sup>2-</sup> would be absent the SO<sub>4</sub><sup>2-</sup> ions, which require a larger hydration shell and are very good at competing with other ions to stay hydrated, would probably instead push the Gdm<sup>+</sup> ions to the surface. One can in Fig. 1(a) also notice a small binding energy shift of about 0.1 eV towards lower binding energy for the spectrum corresponding to the GdmCl + Na<sub>2</sub>SO<sub>4</sub> solution (green dashed) compared to the spectra of GdmCl+NaCl solution and the GdmCl solution without additional ions. The shift towards lower binding energy would then come from the increased electron density due to the doubly charged SO<sub>4</sub><sup>2-</sup> ion at the nitrogen nucleus of the Gdm<sup>+</sup> that in turn would cause the nitrogen 1s electrons to be more loosely bound.

We now know that TPA<sup>+</sup> mainly forms an ion pair with Cl<sup>-</sup> and is pushed to the surface for both solutions with added salt. For the given concentrations of the added salt, the TPA<sup>+</sup>



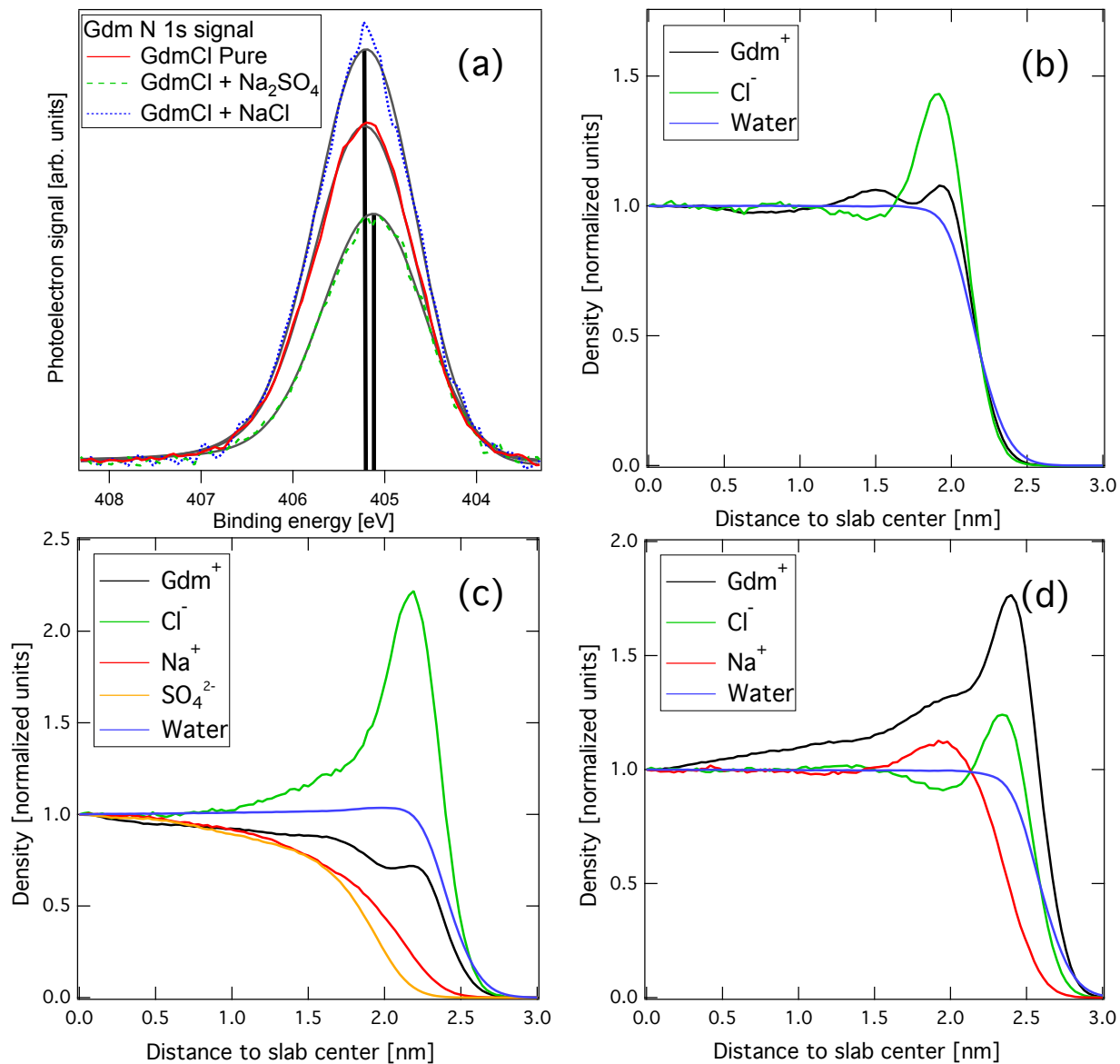


Figure 1: (a) Total intensity XPS spectra of N 1s orbital for the three different GdmCl solutions. (b) Symmetrized number density profiles with the ECC charges of water (blue), guanidinium ion (black), chloride (green), plotted vs. the distance from the slab center and normalized by ion bulk densities at the solution/vapor interface. (c) Similarly as (b) where sodium (red) and sulphate (yellow) is also added. (d) Extra sodium chloride salt is added, note that the Gdm<sup>+</sup> density (black) is higher here compared to (b) and (c).

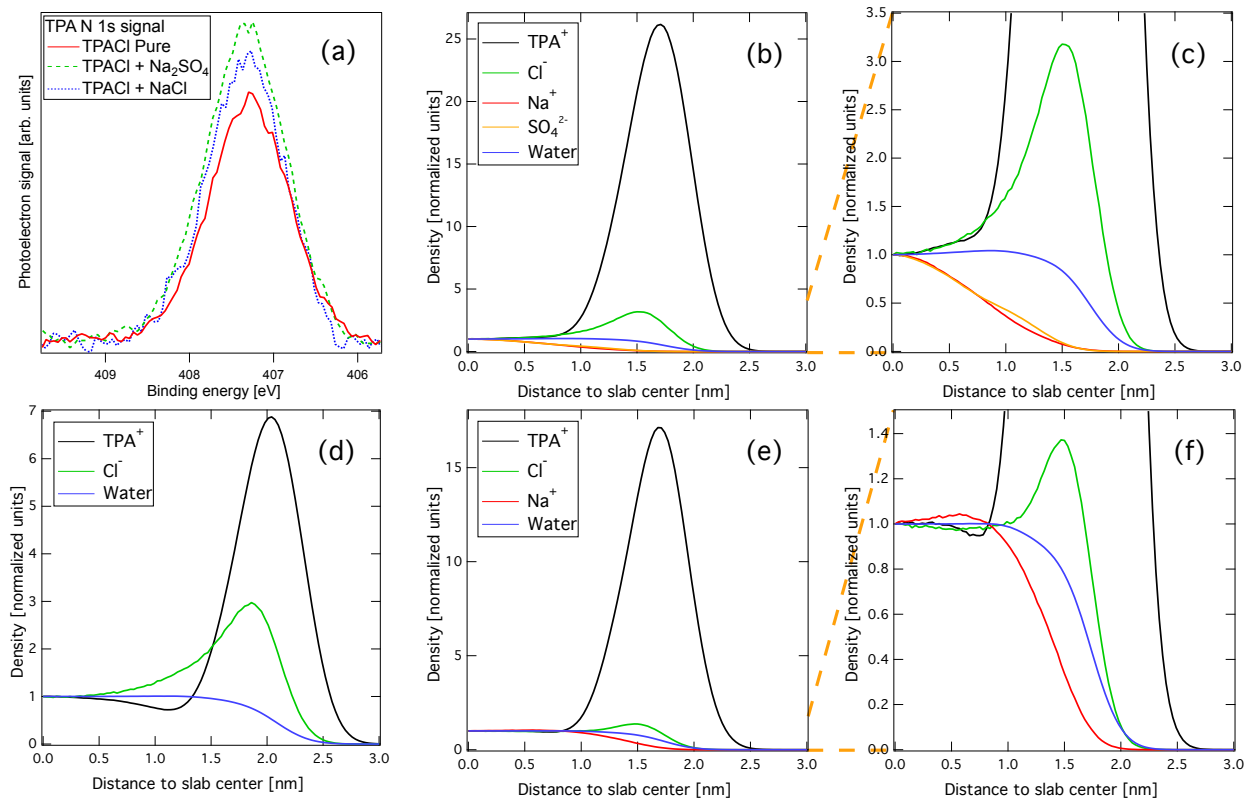


Figure 2: (a) Total intensity XPS spectra of N 1s orbital for the three different TPACl solutions. (b)-(c) Symmetrized number density profiles with the ECC charges of tetrapropylammonium ion (black), chloride (green), sodium (red), sulphate (yellow) and water (blue) plotted vs. the distance from the slab center and normalized by ion bulk densities at the solution/vapor interface. (d) Similarly as (b)-(c) but without  $\text{Na}_2\text{SO}_4$ , notice that the  $\text{TPA}^+$  concentration is lower here. (e)-(f) Extra sodium chloride salt is added.

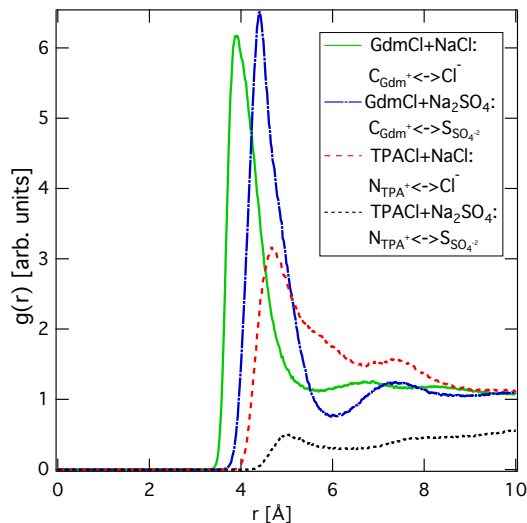


Figure 3: Radial distribution function of the GdmCl+Na<sub>2</sub>SO<sub>4</sub> and TPACl+Na<sub>2</sub>SO<sub>4</sub> respectively.

ions are more efficiently pushed out in the mixture with Na<sub>2</sub>SO<sub>4</sub> compared to the mixture with NaCl. The total concentration of ions are the same in the two solutions, 3 molal of additional ions. Even if there is a difference in the amount of Na<sup>+</sup> added (2 molal to 1.5 molal) the factor that effects the change in TPA<sup>+</sup> surface affinity is most likely dominated by exchanging the SO<sub>4</sub><sup>2-</sup> ions to Cl<sup>-</sup> ions. Again this is mainly due to the difference in the hydration shell size between the SO<sub>4</sub><sup>2-</sup> and the Cl<sup>-</sup> ions - and their ability to stay fully hydrated in the bulk. For the given concentrations the SO<sub>4</sub><sup>2-</sup> ions require, in total, a larger amount of water to form their hydration shells compared to the Cl<sup>-</sup> ions - this is due to the larger physical size and higher charge of SO<sub>4</sub><sup>2-</sup> ions.

In conclusion, this study shows that by adding salts to aqueous solutions surface affinity of solutes will not always increase but can instead decrease. The surface affinity of two different aqueous systems, GdmCl and TPACl, have been studied, where the surface affinity of TPA<sup>+</sup> has been shown to increase with the addition of NaCl and even more when instead adding Na<sub>2</sub>SO<sub>4</sub>, this is explained by that due to the competition of staying hydrated TPA<sup>+</sup> is pushed to the surface. The Gdm<sup>+</sup> solutions exhibit a different behavior in the case of adding Na<sub>2</sub>SO<sub>4</sub>, i.e. the surface affinity of Gdm<sup>+</sup> decreases. MD simulations predicts that

Gdm<sup>+</sup> ions forms an ion pair with the SO<sub>4</sub><sup>2-</sup> ions to a higher degree than the Cl<sup>-</sup> ions in the solution with Na<sub>2</sub>SO<sub>4</sub> added. The SO<sub>4</sub><sup>2-</sup> ions are known to be strongly hydrated and we can conclude that the SO<sub>4</sub><sup>2-</sup>-Gdm<sup>+</sup>-ion pair will reside in the bulk to a higher degree compared to the Gdm<sup>+</sup> ions in the GdmCl solution without additional salt.

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