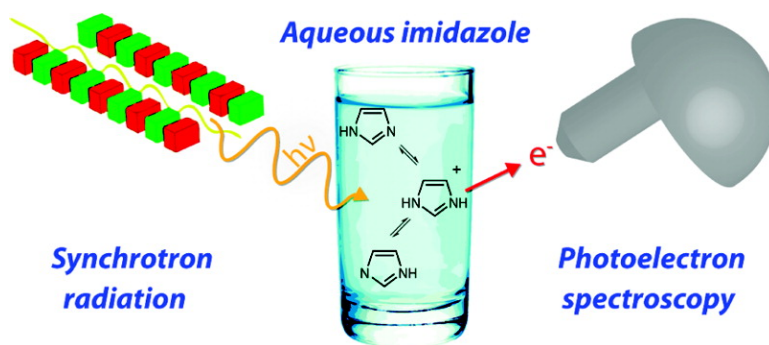## Communication

Pseudoequivalent Nitrogen Atoms in Aqueous Imidazole Distinguished by Chemical Shifts in Photoelectron Spectroscopy

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Pseudoequivalent Nitrogen Atoms in Aqueous Imidazole Distinguished by Chemical Shifts in Photoelectron Spectroscopy

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Rapid (picoseconds[1]) N–H proton exchange between the two nitrogen atoms of neutral imidazole in water is shown here to give rise to distinct binding-energy peaks (chemical shifts) in both the nitrogen 1s and carbon 1s photoelectron spectra. Detection is possible owing to both photoelectron spectroscopy's high sensitivity to local atomic environment and the fast time scale of the photoelectron emission process (approximately 10^{-17} s). In contrast, only one single ^{15}N line is observed at high pH values when using nuclear magnetic resonance (NMR) spectroscopy, which has a time resolution of approximately 10^{-5} s. Thus, in this case the two nitrogen atoms are indistinguable.[2] Nevertheless, both isotropic and anisotropic ^{15}N NMR chemical shifts are good indicators of charge state and hydrogen-bond interactions.[2–4] This was early exploited for microscale titration measurements to determine pK_a values, yielding $pK_a = 6.98$ for imidazole.[5]

Imidazole (Figure 1), a heterocyclic aromatic compound, is part of the side chain of the amino acid histidine, and is the active moiety of many other biological relevant molecules, primarily due to the equilibrium shown in Figure 1, with its pK_a value near neutrality in aqueous solution. Aqueous imidazole is thus a suitable model system for studying pH-dependent molecular hydration and related dynamic properties, for instance in the context with enzyme activity.[6] The considerable protonic mobilities in liquids also make imidazole important in various technological applications, and derivatives of imidazole are often used as cations in ionic liquids.[7]

Photoelectron spectroscopy (PES), ideally in combination with undulator synchrotron radiation, is one of the most powerful experimental techniques to study local electronic structure of matter and provides a completely different access to structure than NMR. Yet PES of aqueous solutions is still a young field, with about a dozen studies published in total. This is due to the high vapor pressure of the aqueous phase, which had hampered the measurement of kinetic energies of the photoelectrons, and only with the recent development of the liquid microjet technique PE spectroscopy under vacuum conditions has become possible.[8] The present liquid-jet photoemission experiments were performed at the soft X-ray undulator beamline U41 (180–1600 eV photon energy, approximately 10^{12} photons/s at a resolution of 1/4000) at the synchrotron radiation facility BESSY, Berlin. The jet diameter was 12 μm, and the liquid-jet velocity was approximately 80 m/s at a temperature of 4 °C. Under these conditions a flowing, free equilibrated liquid-water surface in vacuum is obtained.[8] Electrons were detected normal to both the synchrotron light polarization vector and the propagation of the liquid jet. The experimental setup has been described in detail elsewhere.[8] Imidazole used for
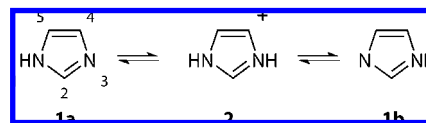

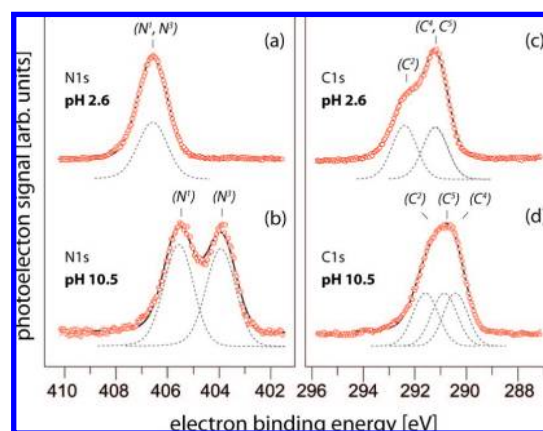**Figure 1.** Lewis structures of neutral (**1a** and **1b**) and cationic (**2**) imidazole.

Figure 2. Nitrogen 1s (a,b) and carbon 1s (c,d) photoelectron spectra (in red) of 2 m aqueous imidazole measured at pH 2.6 (structure **2**) and 10.5 (structures **1a** and **1b**). Photon energy was 480 eV for N 1s, and 380 eV for C 1s measurements. Dashed curves N^i and C^i, are fits, and the thin solid line is the total fit.

preparing the 2 molal solutions was of the highest quality commercially available (>98%, Sigma-Aldrich), and highly demineralized water was used for all experiments. Multimer formation is expected to be negligible given the high solubility of imidazole in water. The pH values of the solutions were adjusted with sulfuric acid at room temperature.

To provide theoretical guidance in the interpretation of the observed spectral changes, energy shifts were calculated for gas-phase imidazole, that is, without solvent interaction. Structures **1** and **2** were optimized using the GAMESS program package[9] at the MP2/aug-cc-pVDZ level of theory. Single-point calculations were performed with the StoBe program package[10] using density functional theory (DFT). To obtain core-level binding energies the Slater transition-state approach[11] was used in combination with a modified gradientless exchange-correlation functional.[12] The TZVP basis set[13] was used for all single-point calculations.

Figure 2, panels a and b show the N 1s PE spectra of imidazole in water at pH 2.6 and 10.5, respectively. The excitation photon energy was 480 eV, amounting to photoelectrons of approximately 75 eV kinetic energy. The latter corresponds to a probing depth in the range of 7–10 Å.[14] No noticeable peak shifts were observed

Table 1. Fitted Experimental and Calculated Electron Binding Energies of Imidazole in Water for pH 2.6 and pH 10.5[a]

	neutral				protonated			
	exptl	$\Delta\varepsilon$	calccd	$\Delta\varepsilon$	exptl	$\Delta\varepsilon$	calcd	$\Delta\varepsilon$
N^1	405.6		406.22		406.6		412.50	
N^3	403.9	1.7	403.95	2.3	406.6	0.0	412.50	0.0
C^2	291.7		291.32		292.4		298.05	
C^4	290.5	0.8	290.72	0.6	291.2	1.2	296.82	1.2
C^5	290.9	0.4	290.30	0.4	291.2	0.0	296.82	0.0

[a] $\Delta\varepsilon$ is the difference between the respective binding energies.

when probing deeper layers. According to the law of mass action, more than 99.9% of all imidazole molecules are protonated at pH 2.6 and deprotonated at pH 10.5, respectively. This explains the occurrence of a single N 1s peak (Figure 2a), at a binding energy (BE) of 406.6 eV, for the pH 2.6 solution, and is consistent with the chemical equivalence of the two nitrogen atoms in the cation **2** due to C_{2v} symmetry. Binding energies were calibrated with reference to the water $1b_1$ peak (11.16 eV[15]), which was measured simultaneously. For cross-check we have also measured the O 1s photoelectron peak of water (538.1 eV[16]) for each solution, using 600 eV photon energy; also the N 1s and C 1s spectra were measured at this energy for reference. All spectra were fitted using a least-squares technique to the postcollision interaction line shape (PCI),[17] which accounts for the interaction between the photoelectron and the Auger electron emitted in the de-excitation of the core−hole state. The line shape was further broadened by a Gaussian distribution to take into account vibrational excitations and Doppler broadening, as well as the finite experimental resolution. The experimental N 1s spectrum at pH 2.6 (Figure 2a) was fitted with two peaks (N^1, N^3) of free but identical width and height, one for each nitrogen atom, that is, assuming similar cross sections for both. The best fit is obtained for the identical peak positions (406.6 eV BE) of both peaks. The situation is entirely different at pH 10.5 (Figure 2b), in which case C_{2v} symmetry is broken. Here, the two pseudoequivalent nitrogen atoms, N^1 and N^3 (of **1a**) give rise to two distinct PE peaks, at 405.6 and 403.9 eV BE, for −NH and −N, respectively. Both peaks are thus appearing at lower energies than observed for the pH 2.6 solution. The spectrum was again fitted with two peaks of free but identical widths, yielding the same Gaussian full-width at half-maximum (fwhm) of 1.2 eV as in the case of pH 2.6. Qualitatively, the observed decrease in N 1s BEs upon deprotonation of imidazole can be associated with the increase of electron density; that is, the missing H atom at N^3 compared to **2** increases the electron density at the nitrogen site, and thus causes a decrease in BE. The 1 eV lower binding energy of the hydrogenated nitrogen, N^1 (for pH 10.5), as compared to N^1, N^3 (for pH 2.6) is due to delocalization of the positive charge across the cation. Experimental energy shifts $\Delta\varepsilon$ are in good agreement with our calculations, shown in Table 1, which contrasts calculated and fitted experimental N 1s as well as C 1s energies for the neutral and cationic imidazole.

Calculated binding energies lead to the same peak assignment as obtained from the qualitative discussion of the electron density. Whereas the absolute BEs are well reproduced for neutral imidazole, absolute values for the protonated species are considerably over-estimated (by approximately 6 eV) due to the absence of the solvent, which shields the positive charge. Energy shifts associated with the change of chemical environment and charge state at a nitrogen site can be also observed in the C 1s PE spectra of aqueous imidazole, presented in Figure 2c,d. For protonated imidazole (Figure 2c), at pH 2.6, one observes a broad peak with a pronounced shoulder at the high binding-energy side. C^4 and C^5 (of **2**) are

chemically equivalent, and accordingly the fit to the spectrum assigns two peaks, C^4, C^5, at the same energy (291.2 eV BE) and a single peak C^2 (292.4 eV BE). Peak positions are summarized in Table 1. Carbon atom C^2 forms two C−N bonds, which reduces the electron density at this site more than for C^4 and C^5. Hence C^2 has a higher C 1s BE, consistent with our calculations.

The C 1s PE spectrum of aqueous neutral imidazole (Figure 2d) is less structured than for the cationic form, reflecting that all carbon atoms are unique, each having a distinct binding energy. Qualitative peak assignment can once again be based on electron density arguments. Upon deprotonation the electron density at the N^3 site (of **1a**) increases, and consistent with observing two distinct N 1s PE peaks for neutral imidazole (Figure 2b), also the carbon atoms C^5 (next to N^1) and C^4 (next to N^3) can be distinguished by their C 1s binding energies. The effect is however substantially smaller for carbon atoms (due to the through-bond interaction). The peak with lowest energy is associated with the higher electron density at the nitrogen site, which is N^3. Hence the fitted peaks with maximum at 291.7, 290.9, and 290.5 eV are attributed to the electron emission from carbons C^2, C^5, and C^4, respectively. This assignment is also corroborated by our calculations (see Table 1). Similarly to the N 1s energies, the C 1s energies are also overestimated in our calculations of gas-phase imidazole, yet the energy shift $\Delta\varepsilon$ correlated with charge delocalization is well reproduced.

We have demonstrated that the two chemically pseudoequivalent nitrogen atoms of imidazole in aqueous solution, resulting from chemical-structural interconversion on the 10^{-11} s time scale, can be distinguished by the N 1s and C 1s peak positions in the photoelectron spectra. The symmetry of the molecules allows a precise determination of the chemical shift induced by a single hydrogen bond (1.7 eV), and of the chemical shift induced by a delocalized charge (1.0 eV). The observed higher N 1s and C 1s binding energies of the protonated imidazole cation are consistent with the complete delocalization of the positive charge. The present study motivates future time-resolved (pump−probe) PE measurements in the aqueous phase.

Supporting Information Available: Complete refs 9 and 10. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Münch, W.; Kreuer, K.-D.; Silvestri, W.; Maier, J.; Seifert, G. *Solid State Ionics* **2001**, *145*, 437.
(2) Munowitz, M.; Bachovchin, W. W.; Herzfeld, J.; Dobson, C. M.; Griffin, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 1192.
(3) Smith, S. O.; Farrjones, S.; Griffin, R. G.; Bachovchin, W. W. *Science* **1989**, *244*, 961.
(4) Wei, Y.; de Dios, A. C.; McDermott, A. E. *J. Am. Chem. Soc.* **1999**, *121*, 10389.
(5) Tanokura, M. *Biochim. Biophys. Acta* **1983**, *742*, 576.
(6) Berg, J. M.; Tymoczko, J. L.; Stryer, L. *Biochemistry*; 5th ed.; W.H. Freeman: New York, 2002.
(7) Wishart, J. F.; Castner, E. W. *J. Phys. Chem. B* **2007**, *111*, 4639.
(8) Winter, B.; Faubel, M. *Chem. Rev.* **2006**, *106*, 1176.
(9) Schmidt, M. W.; et al. *GAMESS 7,* revision 4; 2006.
(10) Hermann, K.; et al. *StoBe-deMon*; version 2.2; 2006.
(11) Slater, J. C.; Johnson, K. H. *Phys. Rev. B* **1972**, *5*, 844.
(12) Proynov, E. I.; Vela, A.; Salahub, D. R. *Phys. Rev. A* **1994**, *50*, 3766.
(13) Godbout, N.; Salahub, D. R.; Andzelm, J.; Wimmer, E. *Can. J. Chem.-Rev. Can. Chim.* **1992**, *70*, 560.
(14) Ottosson, N. Local Ion Distributions at the Vapor/Liquid Interface of Aqueous Alkali-Halide Solutions Measured by Photoelectron Spectroscopy. Master Thesis. Göteborg University, 2007.
(15) Winter, B.; Weber, R.; Widdra, W.; Dittmar, M.; Faubel, M.; Hertel, I. V. *J. Phys. Chem. A* **2004**, *108*, 2625.
(16) Winter, B.; Aziz, E. A.; Hergenhahn, U.; Faubel, M.; Hertel, I. V. *J. Chem. Phys.* **2007**, *126*, 124504.
(17) van der Straten, P.; Morgenstern, R.; Niehaus, A. *Z. Phys. D* **1988**, *8*, 35.

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