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# Simple Atmospheric Hydrogen/Deuterium Exchange Method for Enumeration of Labile Hydrogens by Electrospray Ionization Mass Spectrometry

Yury Kostyukevich,<sup>†,§</sup> Alexey Kononikhin,<sup>†,§</sup> Igor Popov,<sup>‡,§</sup> and Eugene Nikolaev<sup>\*,†,‡,||</sup>

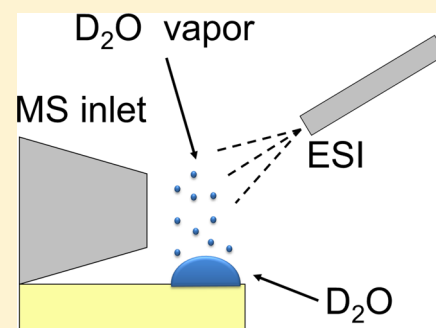
<sup>†</sup>Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Leninskij pr. 38 k.2, 119334 Moscow, Russia

<sup>‡</sup>Institute for Biochemical Physics, Russian Academy of Sciences, Kosygina st. 4, 119334 Moscow, Russia

<sup>§</sup>Moscow Institute of Physics and Technology, 141700 Dolgoprudnyi, Moscow Region, Russia

<sup>||</sup>Orekhovich Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Pogodinskaya st. 10, 119121 Moscow, Russia

**ABSTRACT:** A simple method for hydrogen/deuterium exchange in a standard electrospray (ESI) ionization source is presented. In this method, a D<sub>2</sub>O droplet is placed between the ESI needle and the entrance of the mass spectrometer and thus saturation of the atmosphere with deuterated vapor in the ESI region is achieved. It was shown that full exchange of up to 23 labile acidic hydrogens with a minimal back exchange with the surrounding atmospheric water can be performed by this method.



Hydrogen/deuterium (H/D) exchange is widely used in mass spectrometry for structural studies and investigations of gas-phase reaction mechanisms.<sup>1,2</sup> With dependence on the type of hydrogen being replaced, different exchange reaction techniques are used. Labile hydrogens such as amide, acidic, or hydroxyl can easily be replaced by deuterium in solution, but during infusion into the mass spectrometer a significant back exchange may occur due to interactions with traces of water vapor.<sup>3,4</sup> Hydrogens attached to carbons in the aromatic ring can be exchanged under chemical ionization (CI) conditions in the CI ion source using deuterated reagent gases.<sup>5,6</sup> The depth of the back exchange in the case of labile hydrogens depends on their type. For amide hydrogens, the back exchange can almost be eliminated by infusing nitrogen or dry air into the ion source.<sup>4</sup>

H/D experiments with the most labile acidic and hydroxyl hydrogens can easily be performed in the high vacuum part of the mass spectrometer,<sup>7,8</sup> using collisions with a deuterated gas. Also several successful attempts have been made to perform H/D experiments in the atmospheric pressure ion (API) source region.<sup>9,10</sup> It was shown that in order for the H/D exchange reaction to occur in the API region, a saturated atmosphere of deuterated gas is required. Different experimental designs, like a dual-spray source with D<sub>2</sub>O<sup>9</sup> or a mass spectrometry (MS) interface with curtain ND<sub>3</sub> gas in the capillary-skimmer region, were proposed.<sup>10</sup> However, it is problematic to implement such methods on the standard commercial ESI ion sources without modifications.

Here, we present a simple method to perform hydrogen/deuterium exchange for enumeration of fast exchangeable

hydrogens in compounds in a standard electrospray (ESI) ionization source. The method can easily be performed on any commercial ESI source.

The design of the ESI source for H/D exchange experiments is presented in Figure 1. A copper plate (copper was used for better heat conductivity) was installed just beneath the ESI cone and a droplet of D<sub>2</sub>O was placed on it. The desolvating capillary was maintained at a temperature of 250 °C, and the needle voltage was 2400 V. The temperature of the ESI cone, copper plate, and D<sub>2</sub>O droplet was measured using a thermocouple detector. The temperature of the D<sub>2</sub>O droplet was the same in different parts of the droplet and was equal to 55 °C. The temperature of the ESI cone was equal to 85 °C, and the temperature of the copper plate changed from 85 °C in the points of contact with the ESI cone to 40 °C far from the contact.

Due to the evaporation of the droplet, an atmosphere of D<sub>2</sub>O vapor in the region between the ESI needle and the MS entrance capillary is created. D<sub>2</sub>O molecules can penetrate ESI droplets or participate in ion–molecular reactions inside the desolvating capillary or in the vacuum capillary skimmer region. As a result of these processes, H/D exchange takes place.

Normally, 300–400 μL of D<sub>2</sub>O on the plate was enough for 20 min of experiments. Application of other deuterated liquids such as MeOD and EtOD was also tested, but due to their faster evaporation rate and as a consequence less time for the

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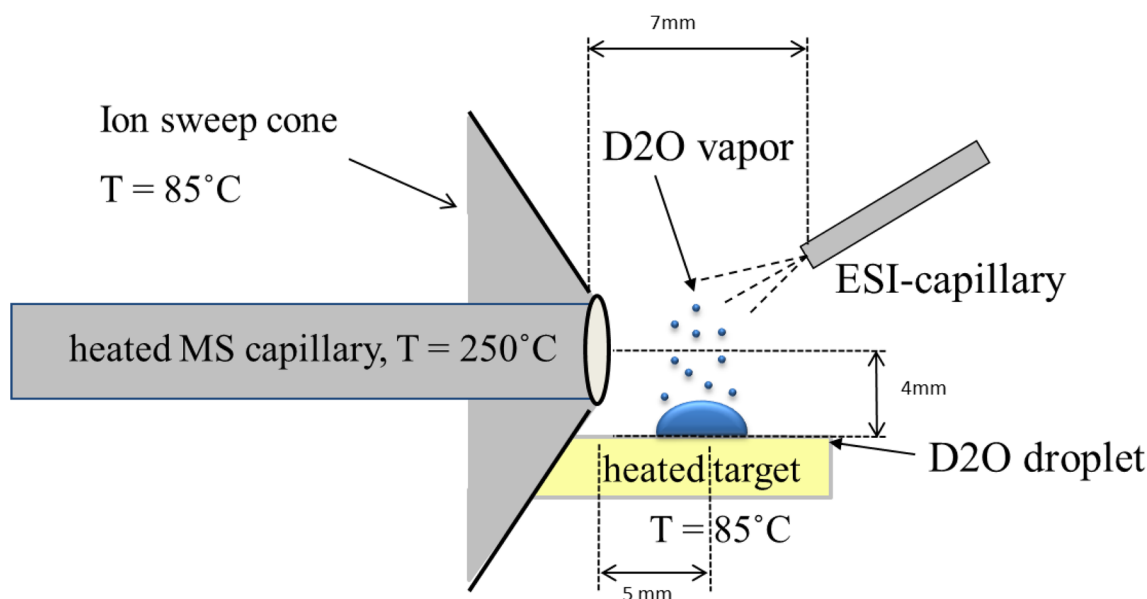


Figure 1. The design of the ESI source modified for H/D exchange experiments.

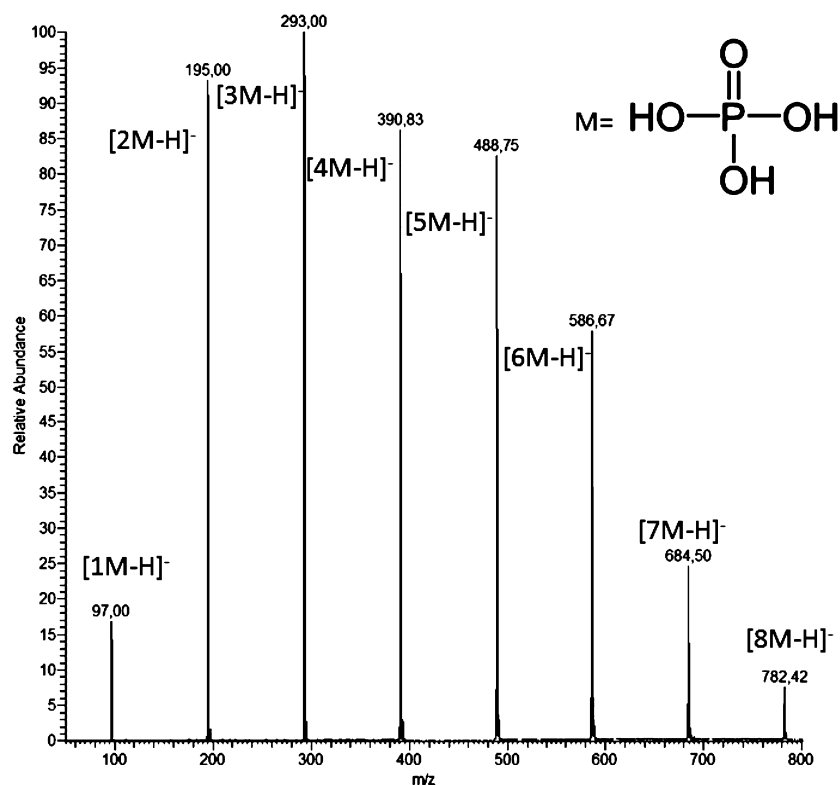


Figure 2. LTQ spectrum of phosphoric acid clusters. Negative ESI mode.

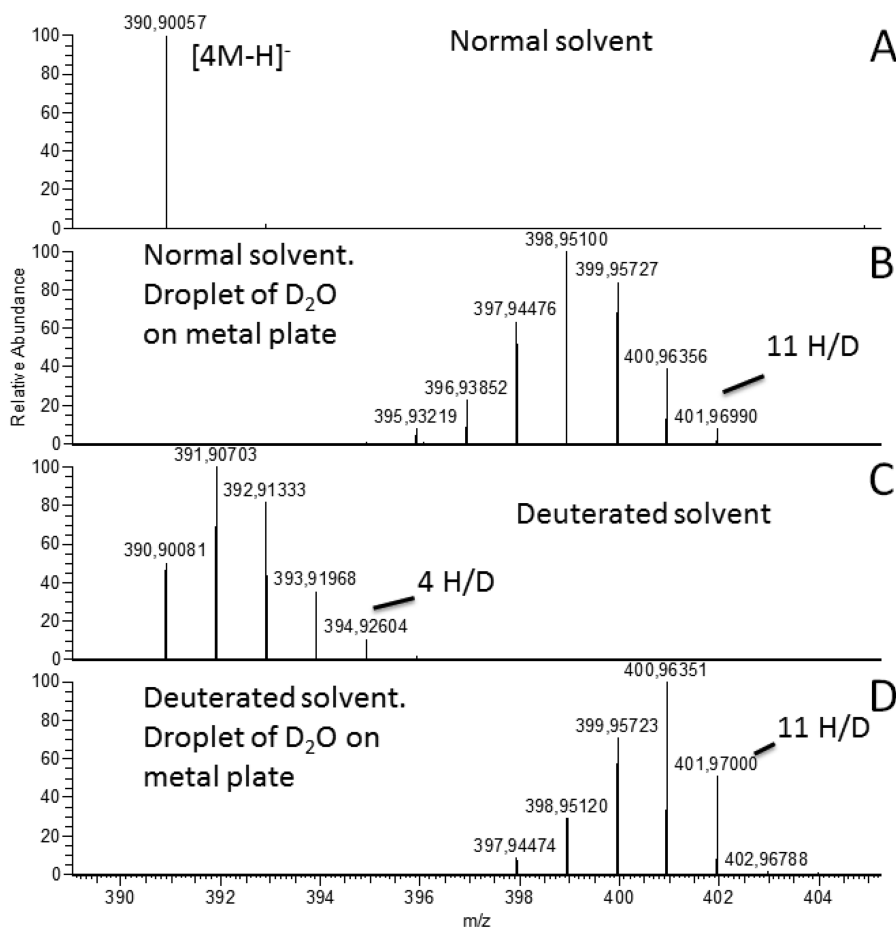
experiment, they seem to be not as good of prospects as deuterating agents.

The spreading area of the D<sub>2</sub>O droplet and mutual distances between the ESI needle, target, and capillary should be chosen that ESI droplets getting into the MS capillary fully interact with D<sub>2</sub>O vapor. Our geometry parameters are shown in Figure 1; the spreading area of the D<sub>2</sub>O droplet was usually 0.5 cm<sup>2</sup>. We did not observe changes in the H/D exchange depth with an increased D<sub>2</sub>O spreading area.

Numerous repetition of experiments demonstrated that the depth of the H/D exchange was the same for different sample infusion rates and amount of D<sub>2</sub>O on the plate.

All spectra were obtained on a Fourier-transform ion cyclotron resonance-mass spectrometer (FTICR-MS) LTQ FT Ultra made by Thermo. Ions were generated in the negative ion mode.

The proposed experimental setup was tested on the ionic clusters formed by phosphoric acid. These clusters cover a large mass range and have many labile acidic hydrogens. The sample preparation procedure was the following: 2 μL of phosphoric



**Figure 3.** FTICR mass spectra of a  $[\text{H}_3\text{PO}_4]_4$  cluster with 11 labile hydrogens. (A) Normal solvent. (B) Normal solvent and a droplet of  $\text{D}_2\text{O}$  placed on the metal plate. (C) Deuterated solvent. (D) Deuterated solvent and a droplet of  $\text{D}_2\text{O}$  on the metal plate.

acid was diluted by 100  $\mu\text{L}$  of water and 100  $\mu\text{L}$  of ethanol and sprayed at a flow rate of 1  $\mu\text{L}/\text{min}$ . For different experiments, normal (nondeuterated) or deuterated solvents were used.

A spectrum of phosphoric acid clusters measured in the negative ESI mode in the Ion Trap of the LTQ FT instrument is demonstrated in Figure 2. Peaks correspond to the  $[\text{H}_3\text{PO}_4]_n-\text{H}^-$  ions. The number of labile hydrogens in cluster  $n$  equals  $3n - 1$ .

In Figures 3 and 4, FTICR spectra of clusters containing 4 and 8 monomers are presented for different experimental conditions described in the legends. The resolving power obtained in these experiments was approximately 300000. It can be seen that the spectra obey the binomial distribution. The relative intensity of the peak corresponding to  $n$  exchanges with depth of exchange  $p$  equals

$$h(n) = C_N^n p^n (1-p)^{N-n} \quad (1)$$

Here,  $N$  is the total number of labile hydrogens. Approximation of the spectra by the binomial distribution (1) allows determining the depths of exchange: (i) Normal Solvent, droplet of  $\text{D}_2\text{O}$  on the plate (71–75%), (ii) Deuterated Solvent (13–17%), and (iii) Deuterated Solvent, droplet of  $\text{D}_2\text{O}$  on the plate (81–85%).

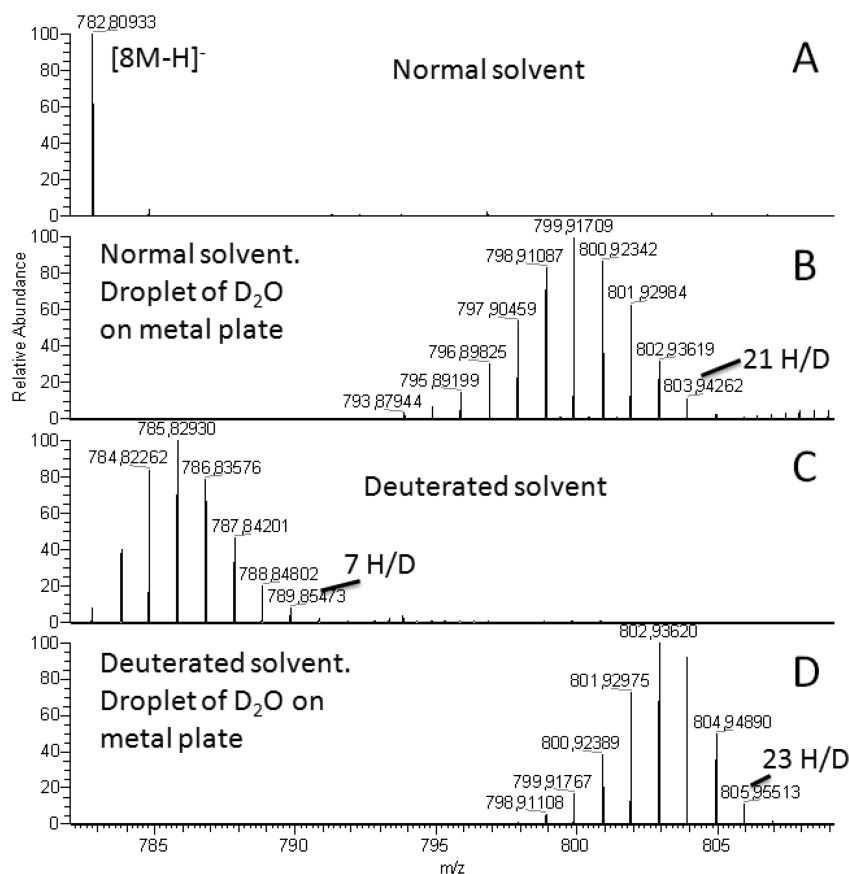
For other clusters, similar results were obtained. The results are in good agreement with previously reported H/D exchange experiments in the ESI ion source.<sup>9,10</sup> The low depth of exchange for the case of deuterated solvent demonstrates the significance of the back exchange in the ESI region due to the

interaction with traces of atmosphere  $\text{D}_2\text{O}$ . Elimination of this effect can be achieved by using deuterated solvent and deuterated atmosphere simultaneously.

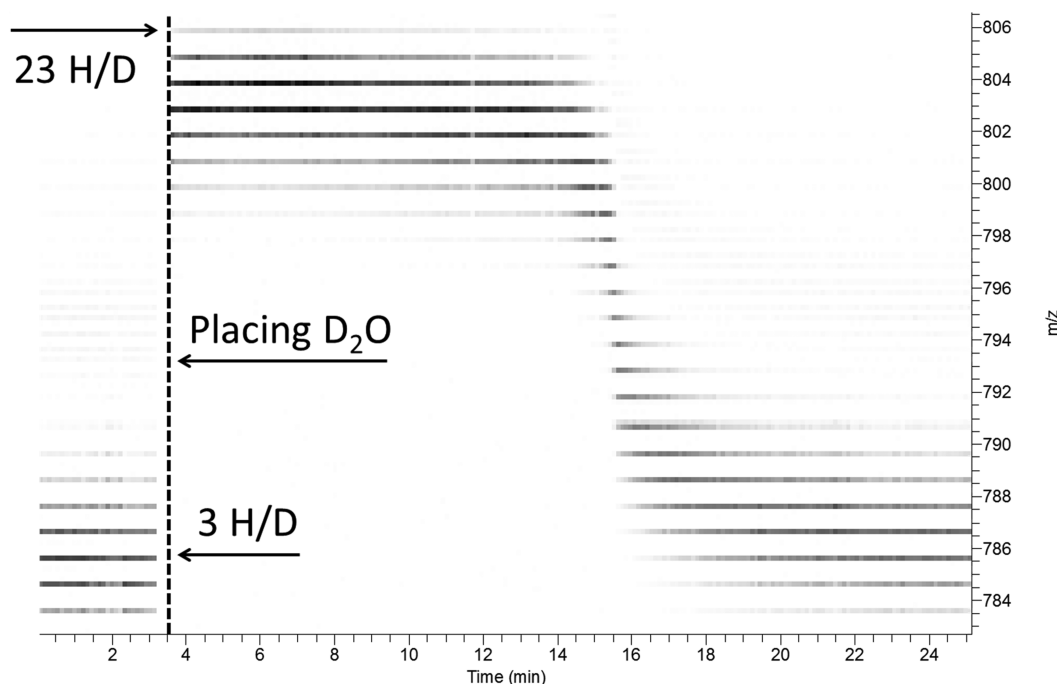
Ultrahigh-resolution FTICR mass spectrometry allows unambiguous identification of the number of labile hydrogens, even in the case of incomplete exchange. In Figure 3D, the binomial distribution breaks off on the right side of the spectrum. The peak corresponding to 11 full exchanges (401.97000) is well-observed. The nondeuterated peak is in the position of  $m/z$  390.90057. If this molecule would have contained more than 11 labile hydrogens, the peak corresponding to 12 exchanges would have had  $m/z$  402.976277 and an intensity comparable to the intensity of the peak 398.95120. But there is no such peak in the spectrum, and thus, it can be concluded that this ionic cluster has exactly 11 labile hydrogens.

Similar considerations work for a twice as big cluster whose spectrum is shown in Figure 4. In Figure 4D, the distribution is wider and the intensity of the peak corresponding to the full H/D exchange is lower. By the same reasoning as with Figure 3D (predicting accurate mass, approximating intensity, and analyzing the spectrum), it can be concluded that the compound has exactly 23 labile hydrogens.

We also investigated the kinetics of the H/D exchange in the API region of the ESI/MS. In Figure 5 is presented an ion current density map for ion cluster  $[\text{H}_3\text{PO}_4]_8$ . The deuterated solvent was used. It can be seen that after the infusion of the  $\text{D}_2\text{O}$  droplet (200  $\mu\text{L}$ ) on the copper plate, it took several seconds to reach the H/D exchange equilibrium. The depth of



**Figure 4.** FTICR mass spectrum of a  $[\text{H}_3\text{PO}_4]_8$  cluster with 23 labile hydrogens. (A) Normal solvent. (B) Normal solvent and a droplet of  $\text{D}_2\text{O}$  placed on the metal plate. (C) Deuterated solvent. (D) Deuterated solvent and a droplet of  $\text{D}_2\text{O}$  on the metal plate.



**Figure 5.** Ion current density map of a  $[\text{H}_3\text{PO}_4]_8$  cluster with 23 labile hydrogens. Black is 100% intensity.

H/D exchange was the same for almost 13 min until the droplet evaporation. After the complete evaporation, the depth of H/D exchange slowly returns to its initial value.

Thus, a method which allows performing H/D exchange in the API region of the ESI/MS interface was developed. It was demonstrated that the saturation of the ionization region by a

deuterating gas can be achieved by evaporating a droplet of D<sub>2</sub>O (or MeOD, EtOD) placed on the sample holder near the entrance of the mass spectrometer. The presented method can easily be implemented on most of ESI ion sources without any changes in the configuration. The method may be useful for structural characterization of different types of organic compounds (carbohydrates, humic acids, etc.) that contain fast exchangeable hydrogens (acidic and hydroxyl).

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: ennikolaev@rambler.ru.

### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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