



## Hydrogen to Deuterium Exchange Rate Measurement in Ionic Liquid-Water Mixture Using NMR

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The rate of hydrogen (H) to deuterium (D) exchange in an aqueous system or bulk water is faster, making it challenging to estimate the rate of H to D exchange using nuclear magnetic resonance spectroscopy (NMR). Confined water behaves differently than bulk water in high viscous media, such as some room temperature ionic liquids and the H/D exchange rate becomes slow, which can be measured *via* NMR. Here, we used selective inversion recovery method to confined water in ionic liquid ([BMIM][BF<sub>4</sub>)]-water-deuterium oxide mixture and measured exchange rate. The numerical value of exchange rate obtained using non-linear curve fitting of the experimental data is 1.34 s<sup>-1</sup>.

**Keywords:** Chemical exchange, Confined water, Ionic liquid, NMR, Inversion recovery.

### INTRODUCTION

Chemical exchange is used in the NMR spectroscopy to describe any process in which a nucleus moves between two or more environments with different NMR parameters (chemical shift, scalar coupling, dipolar coupling and relaxation rate) [1]. Chemical processes causing perturbation of the magnetic environment of atoms are responsible for chemical exchange. It is divided into two types– Intramolecular and Intermolecular. When system whose NMR spectrum shows differences under different conditions, say temperature, then, it is known as intramolecular exchange. Examples include motions of protein side chains, helix-coil transitions of nucleic acids, unfolding of proteins, conformational equilibrium and tautomerization. Intermolecular exchange involves chemical reactions between two or more reactants for example binding of ligands to macromolecules, protonation and deprotonation equilibrium of ionizable groups, isotope exchange processes, enzyme catalyzed reactions, *etc.* [1-3].

A chemical reaction in which a hydrogen atom bonded through covalent bond is swapped by a deuterium atom, or *vice-versa*, is known as hydrogen-deuterium exchange (H–D or H/D exchange) [4-6]. It is most simply applied to exchangeable protons and deuterons, when such a transition occurs

without the use of a catalyst in the presence of a suitable deuterium supply. So long as the substrate is resistant to the conditions and reagents used, the employment of acid, base or metal catalysts in combination with increased temperature and pressure can enable the exchange of non-exchangeable hydrogen atoms [7,8].

Room temperature ionic liquids (RTILs) are ionic liquids that are in a liquid state at room temperature (fusion temperature 100 °C) and largely consist of bigger cations and anions with organic character. They have advantages like comparatively lower vapour pressures, higher mobility of ions under applied electric field, show immunity to fire and stable to heat change [9-12]. RTILs are also known as designer solvents because their properties can be altered by changing the cation/anion combination and they exhibit structural nano-heterogeneity as demonstrated by various studies [13-16]. A study confirmed the existence of two types of domains one is polar and another one non-polar, using simulations on the structure of proto-typical imidazolium based ionic liquids (ILs) [17]. It was also discovered that the polar domain is made up of the imidazolium ring and the anion, whereas the non-polar domain is made up of alkyl chains. The size of nano domain was found to be proportional to the alkyl chain length of the imidazolium ring cation. Later, with the help of molecular dynamics (MD) simulations, structures in

1-octyl-3-methylimidazolium nitrate/water mixtures were reported by Jiang *et al.* [18]. The structure of the original ionic liquid at the nano-domain was found to be intact in mixed solutions where water molecules were situated near the boundaries between the polar and non-polar domains.

A variety of scientific experiments were conducted with the goal of concentrating on the micro atmosphere of water molecules and ionic liquid (IL) cations/anions in order to gain a better understanding of the structure and dynamics of IL/water mixtures [19-25]. With the use of neutron and small angle X-ray scattering, water trapped in the ionic liquid (also known as water pockets) was successfully demonstrated in an aqueous combination containing 80 mol% ionic liquid [26]. Confined water can be found in a variety of systems, including mesoporous silica materials, reverse micelles, chemical and biological systems. Due to the relevance of confined water in a variety of molecular processes, researchers have been interested in studying its structure and behaviour [27,28].

Despite the fact that a great amount of research has been done, direct observations of the state of water contained in ionic liquids are lacking. As a result, no molecular insight into the restricted water was achieved. This was due to a lack of a suitable experimental approach for studying the properties of disordered systems. Because it provides atom-specific information, NMR is a valuable tool for such research. In one investigation, Saihara *et al.* [28] used NMR spectroscopy to directly analyze the status of water in ionic liquids. Because of the dynamic range limitation imposed by the big water peak at these high concentrations, it is usually difficult to generate suitably intense spectra for ionic liquids in aqueous solutions. As a result, while deuterium oxide ( $D_2O$ ) allows NMR spectra of the ionic liquid to be acquired,  $^1H$  NMR analysis of the water peaks is not possible. Water and  $D_2O$  mixed solution were chosen for the testing as a means of resolving the above difficulty [28]. In addition, the use of deuterium hydrogen monoxide (HOD) helped to monitor the exchange of hydrogen and deuterium between dihydrogen monoxide ( $H_2O$ ) and  $D_2O$  occurring inside the water pocket, which is normally not possible to resolve on the NMR timeframe because of the speed of the exchange.

In  $BF_4$ -based ionic liquids, it was discovered that water NMR peaks have two separate resonances with similar chemical shift values, which could be attributed to the existence of two distinct states of confined water, *i.e.* water and HOD [28]. Based on the measurement of the self-diffusion constant, it was deduced that the H and D exchange rate involving the water in the water-pocket was extremely slow.

In present study, the main motive was to measure exchange rate between H/D in water-  $D_2O$ -ionic liquid mixture. Rather than using self-diffusion, which is an indirect measure of chemical exchange, we used NMR methods specifically designed for this purpose to directly assess chemical exchange. The method used here is selective inversion recovery method which involves selectively inverting one of the exchanging signal and observing build-up of another signal of the exchange pair as a function of time, it has been explained nicely by Williams *et al.* [29].

## EXPERIMENTAL

Deuterium oxide ( $D_2O$ ) and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][ $BF_4$ ]) were purchased from Sigma-Aldrich and used as such. Water was used of Millipore grade. All the experiments were performed on Bruker 400 MHz spectrometer equipped with 5 mm BBO probe having Z-gradient and variable temperature unit. Solutions were prepared by dissolving calculated amount of water  $D_2O$  mixture (1:1) in ionic liquid to get samples of varying mol percent from 10 mol percent to 90 mol%. Density of water was taken as 1.00 g/mL and [BMIM][ $BF_4$ ] was 1.21 g/mL. For recording proton NMR spectra temperature was kept constant at 303 K. For attainment of thermal equilibrium, samples were kept for 0.5 h before acquisition.

Proton spectra of samples containing different concentrations of ionic liquid were recorded at different time intervals. These spectra were recorded with and without locking. Spectra were recorded at room temperature and variable temperature. Selective inversion recovery method was used for exchange the rate measurement. Pulse sequence for selective inversion experiment is given in Fig. 1. Data obtained from experiments were fitted using CIFIT [29-31].

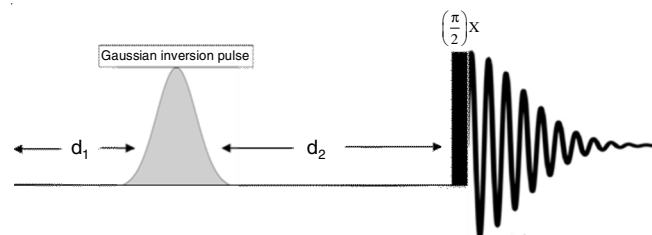


Fig. 1. Pulse sequence used for selective inversion recovery method. Here,  $d_1$  is prescan delay and  $d_2$  is a variable delay

## RESULTS AND DISCUSSION

In pure sample of [BMIM][ $BF_4$ ], we obtained  $^1H$  spectrum (Fig. 2) and peaks were correlated with different protons in structure of the compound, which is shown in picture over the spectrum. It has very small peak at water resonance. Proton NMR spectra of pure [BMIM][ $BF_4$ ] ionic liquid is shown in

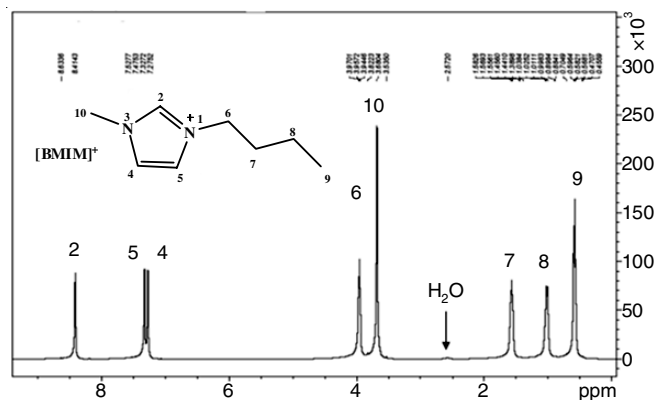


Fig. 2.  $^1H$  NMR spectrum of pure ionic liquid ([BMIM][ $BF_4$ ]) without locking. Different protons are indicated by Arabic numerals over spectra and correlated with structure of ionic liquid cation [BMIM] $^+$

Fig. 2 and different peaks are labeled to their respective proton in the structure of ionic liquid over the spectrum.

If, as suggested by Saihara *et al.* [28] due to nano-heterogeneity in ionic liquid, water is confined in nano domains where rate of exchange (H/D) become slower, we should be able to observe it by NMR spectroscopy. Towards this end, NMR spectra of ionic liquid and water mixtures at varying mole percent were recorded and observed splitting of peak due to D<sub>2</sub>O + H<sub>2</sub>O + HOD mixture, which appears as a single peak in pure water. Proton NMR spectra for 50 mol % [BMIM][BF<sub>4</sub>] was recorded over the temperature range from 30 to 60 °C (Fig. 3). Here, one could see appearance of two peaks at 60 °C and these peaks are also clear in Fig. 4, which is discussed later.

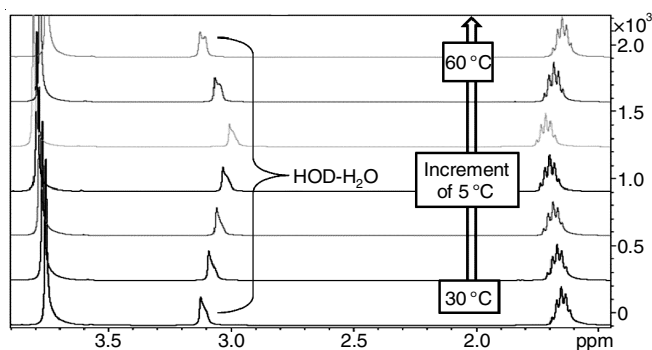


Fig. 3. <sup>1</sup>H NMR spectra of 50 mol% ionic Liquid ([BMIM][BF<sub>4</sub>]) variable temperature. Bottom spectrum at 30 °C and top spectra is acquired at 60 °C increment of 5 °C is used

It is also observed that appearance of two peaks is not same and also unable to reproduce it for same concentration samples run at different times. It is conjectured that this may be due to one of two reasons: due to high viscosity of sample the sample concentration has spatial variation or the magnetic field was inhomogeneous because of spectra being recorded unlocked. Various methods of sample preparation were attempted, such as mixing water only, D<sub>2</sub>O only, D<sub>2</sub>O and water in different layers, *etc.*

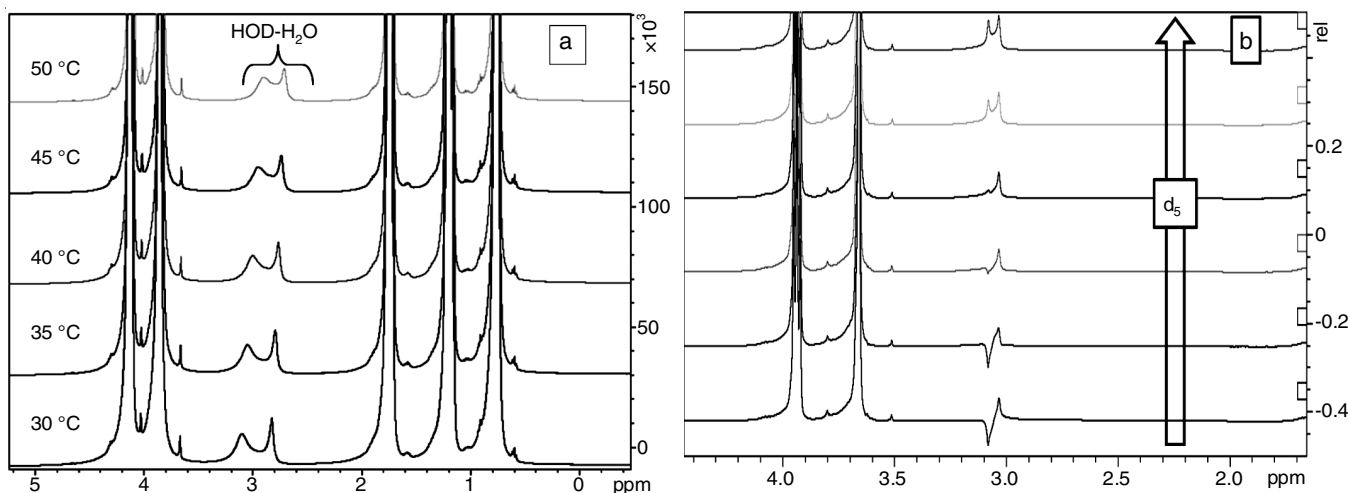


Fig. 4. (a) Appearance of two peaks due to H/D exchange and shift due to temperature change in 80mol% mixture of IL-Water-D<sub>2</sub>O mixture. (b) Overlay spectra of 80 mol% mixture of IL-Water-D<sub>2</sub>O mixture acquired by selective inversion experiment, delay  $d_5$  increases from bottom to top

The appearance of two peaks in the water region is suggestive of a slowdown in the rate of exchange between water D<sub>2</sub>O and HOD, but it was puzzling that the separation between the peaks increases with an increase in temperature; this is counter-intuitive as exchange rates should be higher at higher temperature and peak separation should decrease.

Measurement of the exchange rate by NMR spectroscopy, which was our intent, requires sample and spectrometer stability, especially shimming, for long time, which was difficult to obtain reproducibly. Finally, an external locking system, where one could maintain reproducibility and better shimming was employed. In order to see the effect of water concentration, spectra were recorded at different temperature and composition, which are given below in Table-1.

Mol %	Temp. (°C)	$\delta_{\text{H}_2\text{O}}$ peak (ppm)	$\delta_{\text{HOD}}$ peak (ppm)
80	25	3.13	2.84
	30	3.09	2.82
	35	3.05	2.79
	40	3.00	2.76
	45	2.94	2.73
	50	2.90	2.71

Results in Table-1 shows that with change in temperature one could observe change in the chemical shift value of peak at the resonance of water D<sub>2</sub>O mixture. For comparison, the chemical shifts value for HOD and H<sub>2</sub>O peak were measured with different percentage of ionic liquid and water D<sub>2</sub>O mixture. On changing the composition change in chemical shift values of the water D<sub>2</sub>O peak is observed (Table-2).

Despite lack of confidence in the sample, selective inversion recovery method was applied, in an attempt to measure the exchange rate. The data was obtained using pulse sequence given in Fig. 1. Two peaks, which appeared for H<sub>2</sub>O and HOD in 80 mol% mixture of ionic liquid around 3.13 ppm are shown

TABLE-2  
VARIATION IN CHEMICAL SHIFT VALUES FOR  
WATER-D<sub>2</sub>O PEAKS WITH VARYING Mol PERCENT

Mol % of [BMIM][BF <sub>4</sub> ]	Chemical shift (ppm)
80	3.13
70	3.00
60	3.22
50	3.12
45	2.85
40	2.99
30	2.86

in Fig. 4a and their shift with rise in temperature is also shown. One of these peaks which is shifted more downfield was inverted using selective inversion by G3 pulse. Overlaid spectra are shown in Fig. 4b.

This data was integrated and plotted with increasing delay time  $d_2$ . It is shown below in Fig. 5. With the help of CIFIT, this data was fitted and rate constant was obtained. At room temperature (30 °C) an exchange rate  $1.34 \pm 0.23 \text{ s}^{-1}$  was obtained.

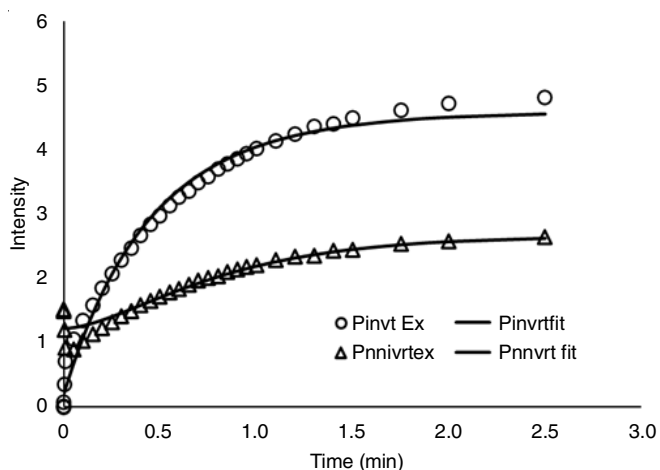


Fig. 5. Non-linear curve fitting plot of selective inversion recovery data. Where Pinvt Ex = Experimental data from inverted peak, Pinvrtfit = Data obtained for inverted peak on fitting, Pnnivrtex = Experimental data from non-inverted peak, Pnnivrt fit = Data obtained for non-inverted peak on fitting

## Conclusion

In a highly viscous medium, the rate constant for hydrogen to deuterium (H/D) exchange was measured. Due to the high viscosity of the ionic liquid, reliable preparation of samples with well-defined concentrations was challenging. It does appear however, that water behaves differently in highly viscous ionic liquid medium. The rate of exchange of hydrogen and deuterium was determined by applying NMR method at room temperature and found to be  $1.34 \text{ s}^{-1}$ . This finding may help to understand the behavioural shift of water present inside water pockets in ionic liquids as compared to bulk phase. Moreover, also help in designing better experimental conditions for studying protein conformation using H/D exchange.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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