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Deep Eutectic Solvent-Based Highly Sensitive Turn-On Fluorescent Probe for D$_2$O

Sushil M. Patil, Santosh Kumar Gupta, Dibakar Goswami,* and Ruma Gupta*

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ABSTRACT: Owing to the importance of heavy water in spectroscopy, nuclear energy generation, chemical characterization, and biological industry, a design of a robust, cheap, nontoxic, and sensitive D$_2$O sensor is very important. In this work, taking advantage of the singular emission fluorescence of the deep eutectic solvent prepared in our laboratory, we propose a first of its kind highly sensitive turn-on fluorescent sensor to effectively sense D$_2$O at an ultratrace level based on rapid exchange of the labile DES proton with deuterium. This method can be used as a full-range heavy water detection strategy with a limit of detection of 0.079% (v/v) or 870 ppm. The isotopic purity (IP) obtained from DES fluorescence measurements is also in close agreement with that of the conventional FT-IR method. The current DES-based sensor thus allows both sensing and isotopic purity of D$_2$O and can serve as one of the most sensitive monitoring strategies for heavy water analysis.
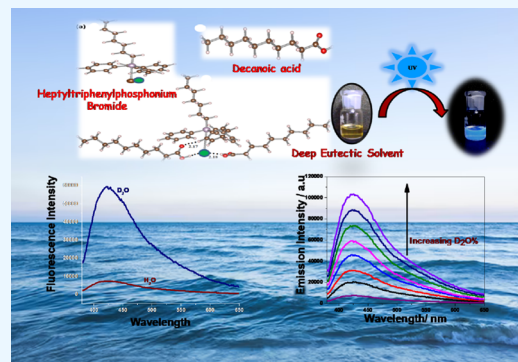
1. INTRODUCTION

High-purity heavy water is used primarily in nuclear power generation as a moderator and as a coolant in pressurized heavy water reactors. Other than this, heavy water plays a very pivotal role in spectral, biological, and chemical analysis.[1] However, D$_2$O is very susceptible to humidity, which affects its purity and severely hampers its practical application.[2] The density of D$_2$O is more than that of H$_2$O, and due to this, drinking heavy water in larger quantities for a prolonged period can cause dizziness and low blood pressure.[3,4] Also, excess D$_2$O over permitted dose induces cell division or mitosis.[5,6] Therefore, it is very important to develop a robust and sustainable method for quantitative detection of heavy water and ascertain its purity. The chemical and physical similarities of D$_2$O to regular water make it difficult to quantify it in the D$_2$O−H$_2$O mixture. The prevailing methods for the quantitative determination of D$_2$O, namely, gas chromatography−mass spectrometry,[7,8] nuclear magnetic spin resonance spectroscopy,[9,10] and Fourier transformed infrared spectroscopy,[11−13] have several drawbacks, such as nonportable setup, need of expensive instrumental design and specialized operators, time-consuming analysis, and complicated operations, leading to inconvenience in rapid point-of-need detection. Apart from that, there are fluorescence-based sensors for heavy water, which have been reported and are listed in Table 1. However, most of them require very cumbersome and time-consuming synthesis, extensive use of organic and toxic solvents, sophisticated instrumentation, etc. as discussed later in the Results and Discussion section.

Deep eutectic solvents (DESs) are a class of solvents formed by mixing quaternary ammonium, phosphonium, or sulfonium salts with a hydrogen bond donor (HBD) in the eutectic molar ratio.[14−17] This further forms a low-melting liquid mixture whose melting point is even lower than that of the individual components, driven by strong hydrogen bonds that help to overcome the lattice energy of the pure components.[14−18] DESs offer a number of advantages, such as low volatility, broad liquid spectrum, low melting points, low/nonflammability, wide electrochemical window, and the ability to be tailored to specific use through judicious selection of ingredients.[17−23] Previously, our group has reported a novel, hydrophobic DES based on heptyltriphenylphosphonium bromide and decanoic acid, which exhibits excellent fluorescence (blue emission) endowed by distortion of phenyl ring pi-electron cloud driven by strong H-bonding between the HBD and hydrogen bond acceptor (HBA) upon excitation at 363 nm with a 23% quantum yield.[24] We envisaged that such a fluorescent, H-bonded system containing a −COOH group may act as an ideal system for D$_2$O sensing, since it is well established that the addition of D$_2$O to a molecule containing functional groups with labile hydrogen, namely, −OH, −COOH, and −NH$_2$, results in a rapid exchange of the labile proton with deuterium, leading to change in its fluorescence properties.[25−29] In fact, once exchanged, the low vibrational
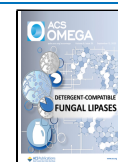
Table 1. Comparison with Fluorescence-Based D₂O Sensors Reported in the Literature

| fluorescent sensor for D$_2$O	inference	refs
coumarin-based small-molecule 6,7-dihydroxycoumarin	low limit of detection (LOD), discriminates between water and D$_2$O in organic solvents	30
	complex synthesis, cumbersome sensing mechanism	
aggregation-induced emission active Eu^{3+}- coordinated polymers	LOD toward D$_2$O in H$_2$O is determined to be 7.8 ppm	31
	high cost factor due to involvement of costly Eu^{3+} ions and cumbersome sensing owing to involvement of multiple fluorescent lines	
phosphorescent MOFs blended with Tb-Gd-Eu blends	cost factor would be very high owing to involvement of three different RE ions	32
	blending multiple RE in MOF will be very difficult to get a uniform and homogeneous solution	
2-[(E)-2-(4-hydroxyphenyl)ethenyl]-1,3,3-trimethyl-3H-indol-1-ium iodide (HTI) (E)-2-(4-methoxystyryl)-1,3,3-trimethyl-3H-indol-1-ium (MTI)	detection limit of D$_2$O = 0.59% (v/v) very complex synthesis method of organic compounds	1
TPA−DP-COOH, a carboxylic acid group-substituted fluorescent substance	low LOD ∼ 49.08 ppm (D$_2$O in H$_2$O) and 0.23 ppm (H$_2$O in D$_2$O), respectively	33
	time-consuming synthesis involving hydrolysis, filtration, evaporation, lots of solvent involvement, not cost effective	
Eu$_{0.4}$Tb$_{0.6}$-MOF	spectral analysis becomes quite tedious involving fluorescence signal from both Tb^{3+} and Eu^{3+}, cost involved in synthesis will be very high due to use of two different rare earth ions, maintaining the stochiometry will be difficult	34
imine-based fluorescent sensor	precise control is needed on hydrolysis reactions to release fluorescent amines and aggregation-induced emission (AIE) of imines	5
deep eutectic solvent	simple preparation, no solvent or any instrumentation requirements, singular fluorescence emission, environmentally benign, highly cost effective with LOD ∼ 0.079% (v/v)/870 ppm	this work
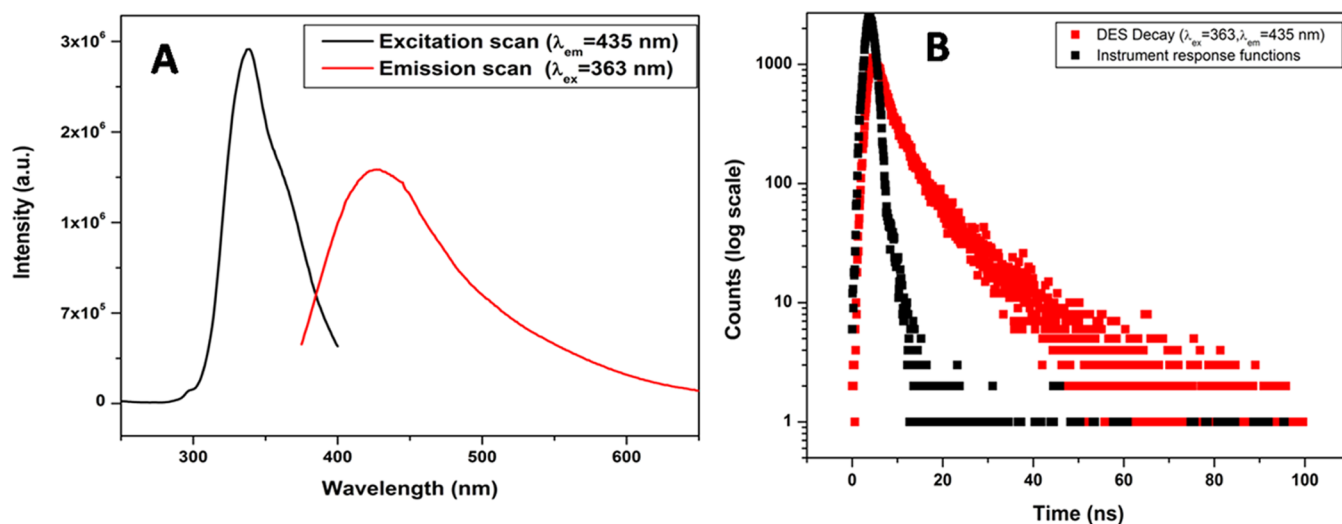
Figure 1. (A) PL excitation and emission spectra of the DES. (B) Fluorescence decay profile of the DES.

energy of the OD bond helps to reduce nonradiative energy losses due to vibrational relaxation, leading to increased radiative power for such luminescent systems with exchangeable labile protons.[25−29] This increase in the fluorescence yield of the DES in the presence of D$_2$O was found to be useful for quantitative measurement of the D$_2$O isotopic purity (IP), and the measurement was found to be at par with the established methods such as Fourier transformed infrared (FT-IR) spectroscopy.

2. MATERIALS AND METHODS

All chemicals except those indicated below were purchased from Sigma-Aldrich and used without further purification. All solvents (AR grade) used for synthesis were purchased from SRL, India, and were dried according to reported procedures. D$_2$O (99.92% pure) was obtained from BRIT, Mumbai, India. The FT-IR spectra were recorded with a BRUKER Tensor II spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with either a Varian 600 MHz NMR spectrometer or a Bruker 800 MHz NMR spectrometer, and the NMR spectra were processed using the Bruker TOPSPIN software or MestReNova-14.0 software. Photoluminescence measurement was carried out on an Edinburgh F900 instrument equipped with a xenon flash lamp of variable frequency (0.1−100 nm).

A DES, based on heptyltriphenylphosphonium bromide [C7, synthesized from triphenylphosphine and 1-bromoheptane, purity ≥99% (HPLC)] and decanoic acid (DA, Sigma-Aldrich, 99%), was prepared following a procedure reported previously by our group[18,24] and was characterized using FT-IR, NMR, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC).[16−18,24] The effect of D$_2$O addition on the fluorescence intensity of the DES was initially optimized by successive addition of D$_2$O [99.92% IP (10−150 μL)] to fresh DES. The fluorescence intensity of the DES was measured after addition of 100 μL of D$_2$O (0−99.92% IP) in 1.5 mL of DES. The mixture was sonicated for 2 mins and was kept standing for another 20 min for equilibration prior to the recording of fluorescence spectra. All spectra were recorded three times to minimize instrumental fluctuations.
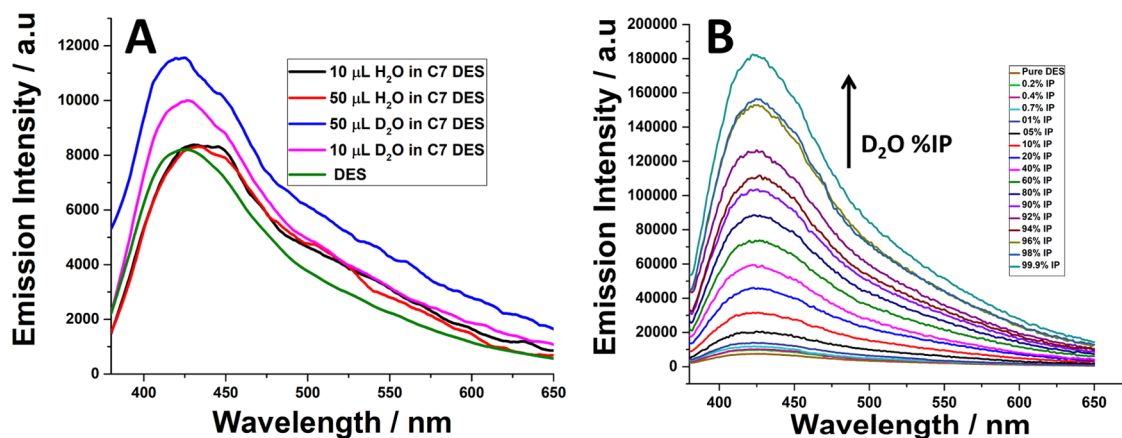
Figure 2. Fluorescence spectra of the DES with (A) either D_2O (99.92%) or H_2O addition and (B) emission intensity (λ 425 nm) of the DES with successive addition of different IPs(%) of D_2O.

3. RESULTS AND DISCUSSION

For a pristine DES, the PL emission spectra (Figure 1A) showed a very intense broad emission band peaking at 425 nm (λ_{ex} = 363 nm), which is attributed to the distortion of the π-electron cloud of the phenyl rings in the DES structure.[16,24] The distortion is a consequence of strong hydrogen bonding ($\geq C=O\cdots H-C_6H_4-$) between the O atoms of the carbonyl oxygen of the decanoic acid and H atoms of the phenyl ring in the C7 salt. The excellent blue emission could be observed directly with the naked eye when placed in a UV chamber (365 nm) [Picture S1, Supporting Information (SI)]. The fluorescence decay (Figure 1B) of the DES at 425 nm emission and 363 nm excitation is biexponential with lifetimes of 2.83 and 10.44 ns with relative amplitudes of 46 and 54%, respectively, giving an average value of 6.94 ns.

The synthesized C7:DA (1:2) DES showed significant fluorescence enhancement when D_2O was added to the system, whereas addition of H_2O had negligible effect on fluorescence intensity, as can be seen in (Figure 2A). The effect of D_2O concentration on the DES fluorescence was further investigated by systematic addition of D_2O with different isotopic purities (0–99.92% D_2O) (Figure 2B). Interestingly, no change in λ_{max} and the characteristics of the spectra suggested that the addition of D_2O to the DES had not caused an irreversible chemical reaction. The ^1H NMR (Figure S1) and the FTIR (Figure S2) spectra of the DES acquired before and after the addition of D_2O provided additional support for this. The quick deuterium exchange with labile hydrogen of decanoic acid, which further depends on the isotopic purity of the supplied D_2O, could be the explanation for the varied fluorescence intensity.

The DES was mixed with different D_2O samples with IPs varying from 0 to 99.92%, equilibrated for 20 min, and the luminescence spectra of the solutions were recorded (λ_{ex} = 363 nm). The correlation between emission intensities at λ 425 nm with %IP of the added D_2O was used to plot the working curve. As shown in Figure 2B, the luminescence intensity increased sharply as the %IP of D_2O increased from 0 to 20%, and there after a slight flat enhancement in fluorescence intensity was observed at higher IPs. This could be caused by the different D-exchange rates owing to different D_2O IPs. When $[(Exp\ (I-I_0))/I]$ was plotted against Ln (%IP of D_2O), the data could be linearly fitted with an R^2 value of 0.9907 (Figure 3). Herein, I and I_0 are the emission intensity at 425
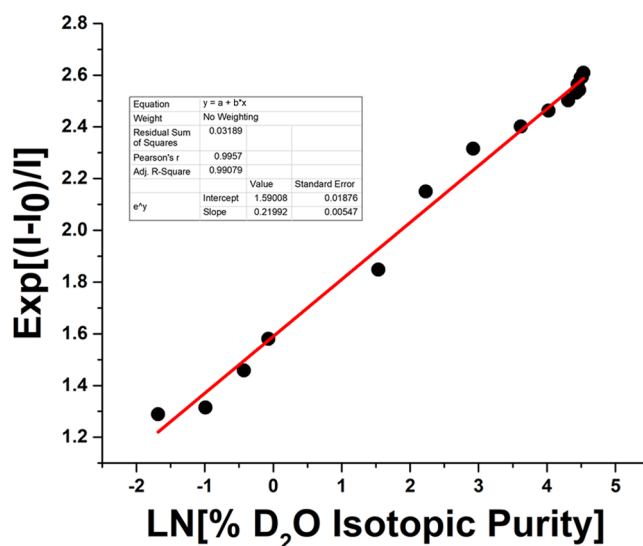

Figure 3. Plot of "Exp (emission intensity at λ 425 nm for x% IP of the D_2O sample (I)) - (emission intensity at λ 425 nm for 0% IP of the D_2O sample (I_0))/(emission intensity at λ 425 nm for x% IP of the D_2O sample (I))" against Ln(%IP of D_2O).

nm for x% IP of the D_2O sample and the emission intensity at (λ 425 nm) for 0% IP of the D_2O sample, respectively. The limit of detection (LOD) was estimated based on the following equation:

$$LOD = 3 \times \sigma/m$$

where σ is the standard deviation of the blank sample and m is the slope of the calibration curve in the region of quantitative heavy water detection.

LOD using our strategy was found to be 0.079% (v/v) or 870 ppm. This low value of LOD renders this method useful as a full-range heavy water detection strategy. The reported LOD is better than 0.595% (v/v) reported by Liu et al.[1]

After the optimization of the fluorescent DES-based D_2O sensing technique, we compared the fluorescent-based sensors for heavy water reported in the literature (Table 1). However, most of the sensors previously reported require very cumbersome and time-consuming synthesis, extensive use of organic and toxic solvents, sophisticated instrumentation, etc. On the contrary, our selfluminescent probe requires simple and cost-effective preparation of the DES from cheap starting
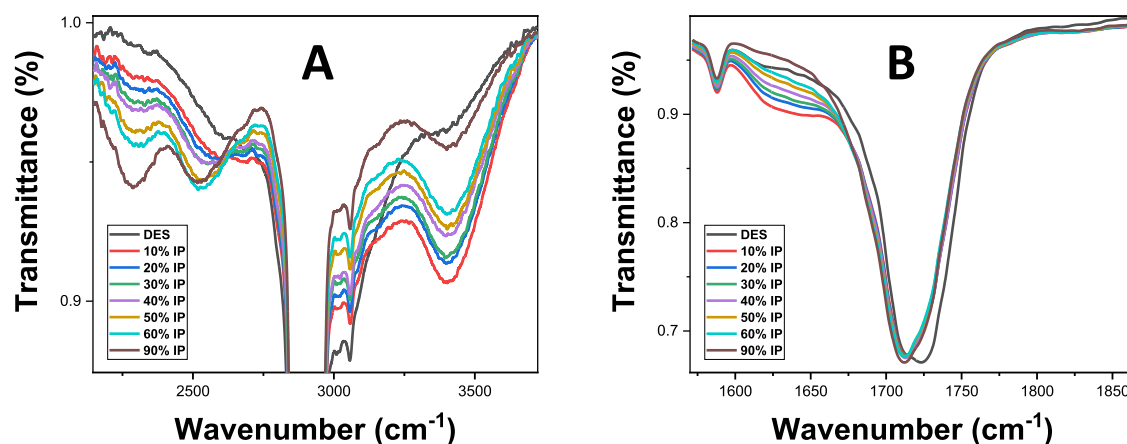
Figure 4. FTIR spectra of the DES with different %IPs of D_2O (A) O−D/O−H stretching peak and (B) carbonyl stretching peak.
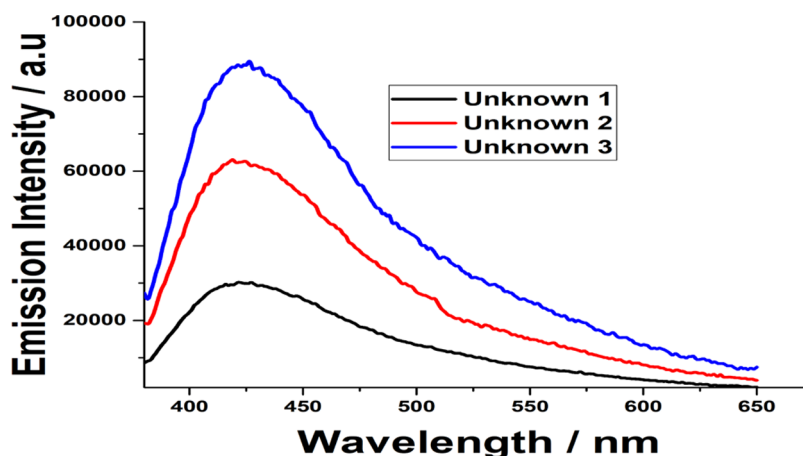

Figure 5. Fluorescence spectra of D_2O samples with unknown %IP.

material with 100% atom economy, thus providing a rapid yet effective tool for heavy water sensing.

4. MECHANISTIC ASPECT

As proposed, the increase of the emission intensity of the DES with an increase in isotopic purities of D_2O is due to the rapid exchange of labile hydrogen on the HBD component (DA) of the DES. It is well established that the vibrational frequency of the O−H bond is more compared to the O−D bond owing to the decreased zero point vibrational energy (ZPVE), as a consequence of heavier mass of D.[35] As was reported earlier, this decrease in vibrational frequency in O−D, in turn, reduces nonradiative energy losses in a fluorophore via vibrational relaxation and promotes fluorescence intensity.[25−29]

The FTIR spectra recorded for each sample with different % IPs of D_2O, presented in (Figure 4a,b), provided evidence of deuterium exchange. The gradual increase in the intensity of the broad peak at 2290 cm^{-1}, corresponding to the stretching vibrations of the O−D bond in −COOD, with a gradual increase in the %IP of D_2O is attributed to the increasing degree of D-exchange. Concomitantly, the intensity of the O−H stretching band in the pure DES gradually decreases, which might be attributed to the D-exchange in the DES. Further, the carbonyl peak at 1723 cm^{-1} of the DES is shifted to a lower energy at around 1713 cm^{-1}, which can be attributed to the decreased C=O bond energy owing to the H/D-exchange in decanoic acid.

In order to utilize the D_2O-dependent increase in the fluorescence intensity of the DES as a quantitative tool to measure %IP of D_2O, a nonlinear exponential plot was fitted using the Bradley equation with a high R^2 value of 0.96758 (Figure S3, SI). This kind of nonlinearity is expected in an isotope exchange phenomenon.[36] This nonlinear dependence of fluorescence intensity is due to the differential rate of deuterium exchange at varying %IPs of D_2O added as well as the D_2O environment in the vicinity of the O−H bond in the HBD plays a part. In the 0−20% range of the isotopic purity of D_2O, the D-exchange occurs in the HBD predominantly, leading to a sharp increase in fluorescence intensity. Thereafter, the increased H_2O concentration (as a result of the exchange) in the surrounding suppresses the increase in fluorescence, leading to a decreased slope, which can be attributed to strong absorption of H_2O in the visible-light region ($\geq$500 nm) due to intense vibrational transitions compared to D_2O.[25,37] When the IP is above 90%, the D_2O concentration surpasses the H_2O concentration, and therefore, a nominal fluorescence enhancement is observed again.

The calibration plot thus obtained (Figure 3) was verified against D_2O samples with unknown %IP, and the fluorescence measurements are depicted in Figure 5. 100 μL of the sample was added to the DES, emission spectra were recorded, and it was fitted in the calibration plot. The %IPs of the samples were also obtained from FT-IR analysis (calibration for FT-IR measurement, see Table S1, SI). The values obtained by our

method were in close agreement with those obtained from FT-IR measurements (conventionally used method) as tabulated in Table 2.

Table 2. Comparison of %IP Values Obtained from Direct FT-IR and DES Fluorescence Measurement

sample	%IP from FT-IR	%IP from fluorescence measurements	[a]RSD
unknown 1	11.50	12.09	±1.18
unknown 2	44.50	44.10	±2.10
unknown 3	64.00	63.75	±1.36

[a]RSD is calculated for triplicate measurements.

5. CONCLUSIONS

A hydrophobic deep eutectic solvent was prepared by mixing heptyl triphenyl phosphonium bromide and decanoic acid as a hydrogen bond donor and acceptor, respectively, in a 1:2 molar ratio, exhibiting a melting point or 19.5 °C. The DES was characterized by NMR and FTIR spectroscopy. The eutectic liquid thus formed exhibited unique photoluminescence properties with broad emission peaking at 425 nm when excited with 363 nm radiation. We further noticed significant photoluminescence enhancement when a small amount of D_2O was added to the DES, whereas addition of H_2O had no effect on luminescence output. Further, the effect of D_2O on the photoluminescence output of the DES was studied systematically to obtain a calibration curve. Thus, by taking advantage of distinguishable photoluminescence spectra with different D_2O contents in the DES, which is not reported earlier for any DES, we were able to quantify the moisture content of D_2O, thus providing a simple, fast, and low-cost sensor for D_2O. FTIR studies provided clear evidence for exchange of labile hydrogen of decanoic acid after D_2O addition, which enhanced photon emission by reducing nonradiative energy losses by vibrational relaxation.

ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02401.

Blue emission of the DES, NMR and FTIR spectra with different D_2O compositions, and FTIR data for standards used for D_2O determination are given (PDF)

AUTHOR INFORMATION

Corresponding Authors

Dibakar Goswami − Bio Organic Division, Bhabha Atomic Research Centre, Mumbai 400085, India; Homi Bhabha National Institute, Mumbai 400094, India; ⓞ orcid.org/0000-0002-4608-767X; Email: dibakarg@barc.gov.in

Ruma Gupta − Fuel Chemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India; Homi Bhabha National Institute, Mumbai 400094, India; ⓞ orcid.org/0000-0003-0136-2399; Email: rumac@barc.gov.in, ruma.chandra@gmail.com

Authors

Sushil M. Patil − Fuel Chemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India; Homi Bhabha National Institute, Mumbai 400094, India

Santosh Kumar Gupta − Radiochemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India; Homi Bhabha National Institute, Mumbai 400094, India; ⓞ orcid.org/0000-0002-1178-0159

Complete contact information is available at:
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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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