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Preparation and Infrared Spectroscopic Studies of Fully Deuterium Oxide Replaced Analogue of Calcium Oxalate Monohydrate

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The present research work reports the preparation of 100% deuterium oxide replaced the analogue of calcium oxalate monohydrate (COM) for the first time. The replacement of crystalline water in COM was carried out in a water-free environment by shaking a portion of the sample of COM with a calculated amount of deuterium oxide. Several experiments were conducted to study both partially deuterium oxide replaced samples and the composition of water/deuterium oxide in the liquid phase. Reflectance spectroscopy was used in analyzing the solid and liquid samples using special sample accessories. Furthermore, a full deuterium oxide replaced sample was exposed to atmospheric water to study the exchange of deuterium oxide molecules from the solid samples with water molecules. The results show that the full deuterium oxide replaced COM can be prepared using a suitable water-free environment. The infrared spectra of these samples clearly show that the water molecules in the crystal structure were replaced by deuterium oxide molecules assuming identical positions in the crystal structure. However, the bands arising from the deuterium oxide molecules were narrower than the bands due to water molecules. The analysis of the liquid samples during the partial deuterium oxide replacement shows that the replacement of the water of crystallization by deuterium oxide is 60% after 6 h of shaking. The full deuterium oxide replaced sample exposed to atmospheric water shows that the replacement of deuterium oxide by water molecules from the vapour phase is a slow process.

Keywords: Calcium oxalate monohydrate, Deuterium oxide, Infrared, Diffuse reflectance, Kubelka-Munk, Heterogeneous phase.

INTRODUCTION

Calcium oxalate monohydrate ($CaC_2O_4{\cdot}H_2O$, COM) is known as whewellite not only found as a mineral but, also found in plants, urinary calculi and other biological objects [1]. COM aroused the research interest, as it is a most common constituent of urinary calculi. Approximately 75% of all stones contain calcium oxalate and an additional 5% are composed of calcium phosphate. There are two forms of calcium oxalate in urinary calculi; COM and calcium oxalate dihydrate (COD) [2-4].

The crystal structure of COM contains two nonequivalent oxalate ions thus forming two different environments for water molecules namely Type 1 and Type 2 water molecules as shown in Fig. 1. These two different water molecules in the crystal structure have long and short hydrogen bondings with oxygen atoms of the acidic carbonyl groups of oxalate ions. Infrared peak assignments for the OH stretchings of these two different water molecules are given in Table-1. The peaks at 3486 and 3058 cm^{-1} are due to Type 2 water molecules and 3428 and 3336 cm^{-1} are due to Type 1 water molecules [1]. The fifth peak at 3258 cm^{-1} is due to the overtone of HOH bending vibration reinforced by the Fermi-resonance of Type 2 water molecules [6].

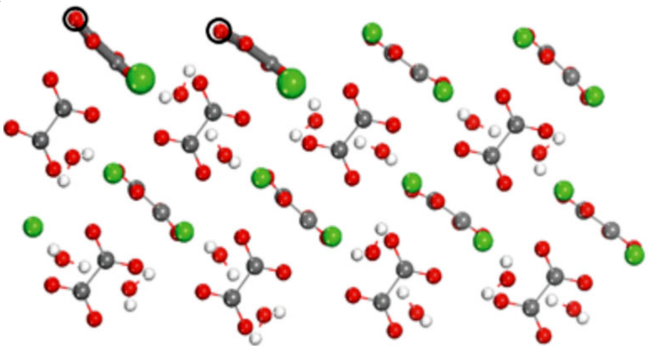

Fig. 1. Structure of the calcium oxalate monohydrate (reproduced from [Ref. 5]). Ca^{2+} ion in green, carbon in black, oxygen in red and hydrogen in white

TABLE-1
INFRARED BAND ASSIGNMENTS OF THE
O–H STRETCHING VIBRATIONS IN WATER [Ref. 1]

$CaC_2O_4 \cdot H_2O$ absorption (cm^{-1})	Assignment
3486	ν(OH)(2)
3428	ν(OH)(1)
3336	ν(OH)(1)
3250	2δ(OH)
3058	ν(OH)(2)

The dehydration of these two types of water molecules takes place in two steps [6]. The first water release step takes place between 90 to 125 ºC, while the second water release step takes place between 125 to 150 ºC. These confirm the presence of two different environments for the water molecules in the COM crystal structure. Although the dehydration of both types of water molecules takes place at the same time the rates of dehydration are different. Type 1 water molecules are attached to the crystal structure with weaker hydrogen bondings compared with Type 2 water molecules. Therefore, Type 1 water molecules start leaving the crystal structure first during thermal decomposition [6].

The crystal structure of COM has been studied by several authors [7,8]. Cocco [8] was the first researcher to investigate the data of this structure, whereas further detailed analysis was done by Tazzoli *et al.* [9] and Deganello *et al.* [10]. Trpkovska *et al.* [2] were the first to report the spectral data of the structure of COM at the boiling temperature of nitrogen.

In addition to the studies on the crystal structure of monohydrate, researchers have paid attention to the formation of its D_2O analogue. Nevertheless, they were unable to prepare a fully deuterated analogue of COM. Petrov & Sopatrajanov [1] were the first to study the partially deuterated COM. Thereafter, due to the availability of superior instrumentation and other improved experimental and theoretical opportunities, Trpkovska *et al.* [2] studied the FTIR spectrum of whewellite and some of its deuterated analogues at low temperatures. Surprisingly, we have successfully obtained the first completely D_2O-substituted analogue of COM. This allowed us to study and compare the spectra of these two analogues using infrared spectroscopy. This work was focused on the conditions needed for the preparation of 100% D_2O replaced COM, the progress of D_2O exchange with water of crystallization in COM and the behaviour of fully D_2O replaced COM when exposed to moisture in the surrounding room temperature.

EXPERIMENTAL

Calcium oxalate monohydrate ($CaC_2O_4 \cdot H_2O$, COM) (> 98%) purchased from Sigma-Aldrich was used after evacuating at 80 ºC for 30 min to remove water absorbed by physisorption [6]. Doubly distilled water and D_2O purchased from Sigma-Aldrich (99%) were used in the experiment.

A ceramic heater (BA electric Bunsen from Electrothermal, UK) controlled by an external power supply was used to heat the samples. A digital thermometer with a K-type thermocouple (Clasohlson, U.K.) was used to measure the temperature of the samples. For the evacuation of the samples, a powerful vacuum pump (Edwards, U.K.) with a pressure of 10^{-2} bar was used. A MS 1 mini-shaker IKA was used at a rate of 400 min^{-1} to shake the samples. A chromabond standard development kit containing SB (SAX) was bought from Analytichem International to extract only the liquid phase from COM and D_2O mixture. The cartridge in the development kit had a volume of 3 mL and 500 mg SPE material packed at the bottom of the cartridge.

FTIR analysis: A Perkin-Elmer spectrum one NTS spectrometer (Perkin-Elmer Ltd., U.K.) equipped with a transflectance accessory and deuterated triglycine sulphate detector was used in measuring the infrared spectra in the near infrared region. The COM samples were preheated and evacuated at 80 ºC for 30 min in glass vials to remove the adsorbed water [6]. Then the sample bottle containing the COM was placed directly on the crystal of the transflectance accessory and the near infrared spectrum of sample was measured in the region of 10,000 to 4,000 cm^{-1} at a resolution of 16 cm^{-1}. A total of 20 scans were made during each NIR spectral measurement.

The samples for mid-infrared analysis were preheated at 80 ºC for 30 min to remove adsorbed water (or D_2O) molecules [6]. Each of the water-free samples was then prepared as 10% sample mixtures (w/w) in finely ground dry KBr. A Perkin-Elmer spectrum one FT-IR spectrometer equipped with a medium band MCT detector and diffuse reflectance accessory (Perkin-Elmer) was used to acquire the infrared spectra in the Mid-IR region. A total of 32 scans were made each time in the range of 4000 to 650 cm^{-1} at a resolution of 8 cm^{-1}. When analyzing the solid samples using diffuse reflectance accessory a background spectrum of KBr was measured under the same conditions of the experiment. The infrared spectra measured on the series were saved as absorption spectra. Each infrared absorbance spectrum was then derivated and their second derivative profiles were saved for further analysis.

Preparation of partially D_2O replaced COM

Displacement of H_2O in $CaC_2O_4 \cdot H_2O$ by D_2O: During the preliminary investigation of the replacement of water molecules in COM by D_2O, the COM was allowed to be in contact with D_2O at different times as described below. A small portion of $CaC_2O_4 \cdot H_2O$ was taken into a sample bottle and then evacuated at 80 ºC for 30 min to remove physically absorbed water from it [6]. Thereafter, $CaC_2O_4 \cdot H_2O$ (0.8018 g, 0.0055 mol) was treated with D_2O (0.9781 g, 0.0489 mol) in a syringe with SAX sorbent and continuously shaken for 1 h [11]. The same was repeated for 3 and 6 h (Fig. 2). Then the liquid in the syringe was eluted using a vacuum elute. The SAX material retains oxalate ions and the elute contains only the mixture of H_2O and D_2O. The resulting liquid mixture was analyzed using the FT-IR spectrometer with a single reflection attenuated total internal reflectance accessory and a medium band MCT detector.

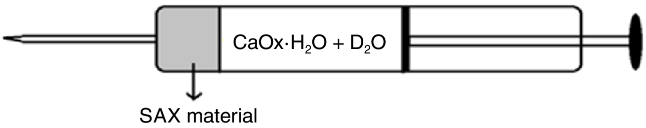

Fig. 2. Experimental setup for the displacement of H_2O in $CaC_2O_4 \cdot H_2O$ by D_2O

Preparation of fully deuterated analogue of $CaC_2O_4·H_2O$: The sample bottle containing COM was enclosed using a septum with an aluminum cap where a small hole is made to insert the glass tube with a stopper. The glass bottle was sealed with an aluminum cap to create a water-free environment as shown in Fig. 3. The sample was then evacuated at 200 ºC for 2 h [6]. The calcium oxalate obtained by evacuation was cooled and D_2O was injected through a syringe into the sample bottle without disturbing the water-free environment as the septum has a fascinating feature of closing back again. Then, it was continuously shaken for 6 h and kept for two nights. Then the sample bottle was evacuated at 80 ºC for 30 min to remove surface D_2O. The resulting sample was analyzed using FT-IR spectrometer equipped with a diffuse reflectance accessory.

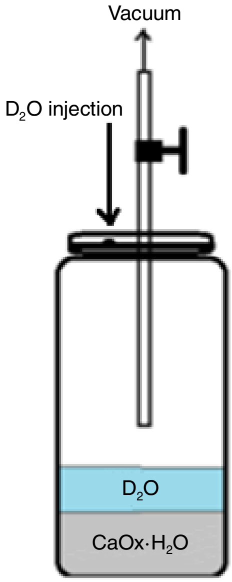

Fig. 3. Experimental setup for the preparation of deuterated analogue of COM

Displacement of D_2O in $CaC_2O_4·D_2O$ by atmospheric H_2O: The D_2O replaced analogue of COM was tested for its exchange with water molecules in the atmosphere to study the reaction progress. A mixture of finely ground KBr (Merck) and $CaC_2O_4·D_2O$ (10% w/w) was made by mixing weighed amounts of $CaC_2O_4·D_2O$ and KBr. Then the sample was placed in a sample cup and levelled with a spatula. Thereafter, it was exposed to water in the atmosphere (at 52% humidity) to facilitate the displacement of D_2O for about 8 consecutive days. Every time a background spectrum of KBr was measured before measuring the spectrum of the sample. Each sample spectrum was transformed into Kubelka-Munk format [12] and saved again for further analysis.

Calibration: The composition of the liquid eluted after the exchange of water molecules in COM with D_2O was determined by using an external calibration plot of mixtures of water and D_2O. A glass tube of 3 cm height and 8 mm diameter was connected above the ATR crystal as shown in Fig. 4. The background of the system was scanned without any content in it. A small amount of water sample was drawn using a syringe and the total weight was determined by an analytical balance. The water sample was injected carefully into the glass tube and the empty syringe was weighed again. The amount of water in the tube was then determined by the difference in weights. Small portions of D_2O were injected into the tube containing water. The amount of D_2O added was determined in the same manner as described above and each time the spectrum of the mixture was recorded. The calibration cannot be conducted the other way around (adding water to D_2O) because the mixing of the two liquids is not effective due to their density difference [13].

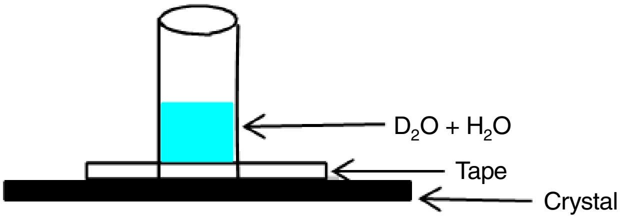

Fig. 4. Setup for calibration

RESULTS AND DISCUSSION

Interpretation of IR spectroscopy of COM and D_2O analogue: The Kubelka-Munk format of the diffuse reflectance infrared spectrum of COM is illustrated in Fig. 5. The –OH stretching of the water molecules in the COM crystal structure appear between 3500 to 3000 cm^{-1} [1-3,6]. The spectrum depicts five distinct bands in the stretching area. According to the former studies [1-3,6], the COM crystal contains two types of water molecules: Type 1 and Type 2. Christy *et al.* [6] already assigned these five bands *viz.* 3428 and 3336 cm^{-1} from OH stretching of Type 1 water molecules, 3486 and 3058 cm^{-1} from OH stretching of Type 2 water molecules and the weak band at 3250 cm^{-1} is due to the overtone of the bending vibration of Type 2 water molecules reinforced by Fermi resonance. These details were studied using the dehydration profile of COM. Though Petrov *et al.* [1] mentioned the five bands of OH vibrations in COM at 3495, 3440, 3340, 3250 and 3060 cm^{-1}, however, failed to assign the bands as Type 1 and Type 2.

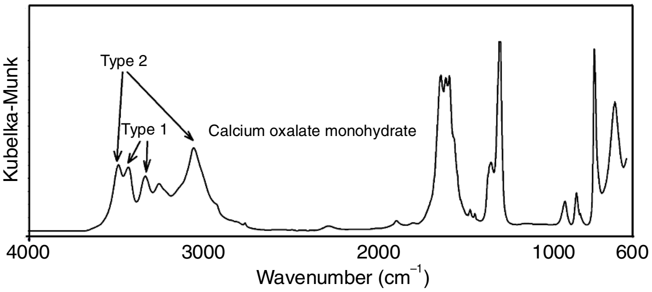

Fig. 5. Diffuse reflectance infrared spectrum of 10%(w/w) of COM in KBr

The five characteristic H-O-H stretching bands of the crystal water in calcium oxalate are further confirmed by the diffuse reflectance infrared spectrum of 10% (w/w) of COM in KBr as shown in Fig. 6. The bands appeared at 3488, 3434, 3336, 3259 and 3059 cm^{-1} are in good agreement with bands obtained by both Petrov [1] and Christy *et al.* [6]. There is no doubt, however, that the oxalate stretching bands are more intense than the OH ones.

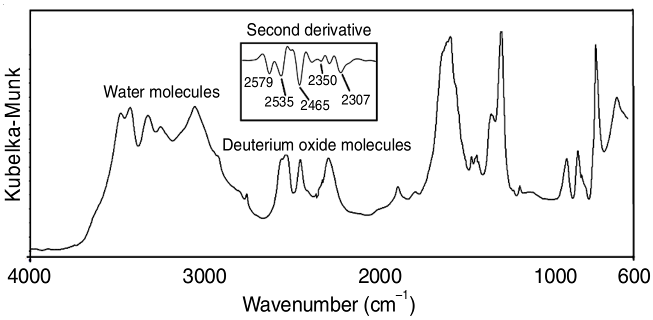

Fig. 6. Diffuse reflectance infrared spectrum of 10%(w/w) of COM after 6 h of treatment with deuterium oxide in KBr

The infrared spectrum of the partial deuterium oxide replaced COM for 6 h is shown in Fig. 6. Variation in spectral profiles of the diffuse reflectance infrared spectra of COM and its partially deuterated analogue was analyzed. The OH stretching bands of water molecules in COM in the partially D_2O replaced sample have been slightly shifted. Only three bands are visible for OD stretchings of D_2O molecules in the sample. However, the second derivative profile of the spectral profiles shows five bands at 2579, 2534, 2464, 2378 and 2306 cm^{-1} (Fig. 6). The bands recorded in the previous study addressed by Petrov *et al.* [1] were in agreement with these bands to some extent. All the five bands are clear in the fully D_2O-replaced sample. However, these bands experience a blue shift as the percentage of D_2O displacement increases. The displacement of H_2O by D_2O is a continuous process. Fig. 7 shows the diffuse reflectance spectrum of COM treated with D_2O after 1, 3 and 6 h. The displacement of water molecules by D_2O molecules is evident from the increase in the intensities of bands representing the OD stretchings of the D_2O molecules in the crystal.

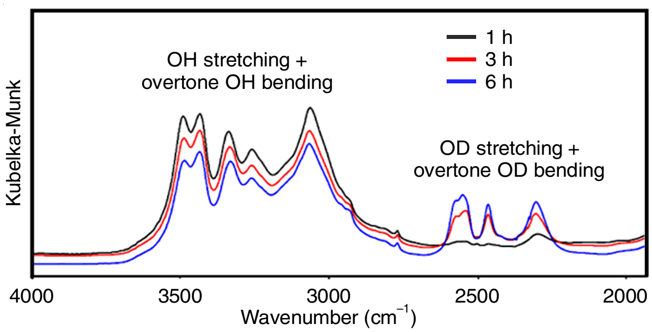

Fig. 7. Diffuse reflectance spectra of COM treated with D_2O in 1, 3 and 6 h 10% (w/w) in KBr

As mentioned earlier the quantification of D_2O displacement was carried out using an external calibration. It is important to remove the contaminated oxalate ions from the liquid eluted after the exchange reactions to determine the extent of D_2O replacement in the samples. The fundamental HOH bending band (at 1643 cm^{-1}) always seems to be hidden under an intense oxalate band at 1620 cm^{-1} [1,2]. Distinguishing one another was an extreme challenge for the scientists who studied this earlier. However, we managed to overcome this by analyzing the liquid sample without the interference of oxalate bands by eluting through a SAX sorbent.

Fig. 8 clearly shows three distinct peaks in the range of 1700 to 700 cm^{-1}. The peaks acquired at 1643, 1454 and 1208 cm^{-1} represent H-O-H, H-O-D and D-O-D bending modes, respectively. Previous research studies conducted by Christy *et al.* [13] and Petrov *et al.* [1] were in agreement with the above peaks. The liquid mixture eluted through the SAX column had no oxalate peak and the interference of oxalate peak was avoided in the quantification of H_2O and D_2O in the liquid mixture.

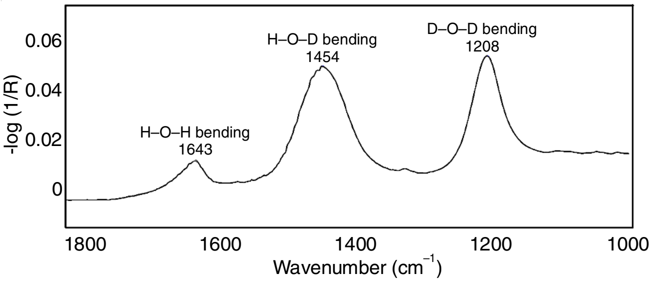

Fig. 8. Mid infrared spectrum of liquid sample taken after treating COM with D_2O for 6 h

To find out the amount of water that has been displaced by deuterium oxide; a multivariate calibration was constructed by using infrared spectral profiles obtained in the region 1800-1000 cm $^{-1}$ for the calibration mixtures by adding D_2O to preweighed H_2O. When D_2O was added to water, the mixture quickly establishes an equilibrium as shown below. It means that the liquid eluted after an exchange reaction of COM with D_2O will contain H_2O, D_2O and HOD.

$$H_2O + D_2O \rightleftharpoons 2HOD$$

The calibration model so established was used to predict H_2O in the eluted mixture and then the percentage of water molecules displaced was calculated. The calculation shows that around 60% of water in COM has been displaced by deuterium oxide at the end of 6 h (at 22 ºC).

Interpretation of IR spectroscopy of fully deuterated analogue of COM: The preparation of fully deuterated analogue was a challenge. Dry COM sample was used in the exchange with D_2O. The presence of adsorbed water was monitored by NIR spectroscopy. Adsorbed water formed a peak at 5200 cm^{-1}, which was eliminated by heating the material under vacuum.

The diffuse reflectance spectrum of the fully deuterated analogue of COM sample is shown in Fig. 9. The spectrum clearly shows the absence of water molecules in the crystal structure and confirms that COM can be fully deuterated by treating it with D_2O in a water-free environment. Fascinatingly, it was revealed from the shape of peak that the fully deuterated analogue of COM to displays a crystalline nature. Five sharp peaks were observed between 2600 to 2200 cm^{-1} according to the literature [1,2,13]. There are five main bands appearing in the spectrum at 2584, 2551, 2465, 2398 and 2297 cm^{-1}. The exact peak positions observed here seem to be shifted when compared with the peaks observed for the O-D stretching in a partially deuterated analogue. Since this study compared fully deuterated and partially deuterated samples, these variations were to be expected. The spectral patterns of the OH and OD

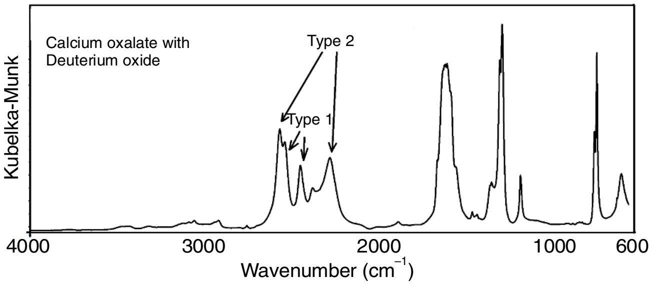

Fig. 9. Diffuse reflectance infrared spectrum of fully deuterated analogue of COM 10% (w/w)

stretchings in the crystal structures show that the OD stretching bands are narrower (2700-2100 cm^{-1}) compared to OH stretching bands (3800-2600 cm^{-1}). The OH stretching bands span over a range of 1200 cm^{-1} compared to OD stretching bands that span over a range of 600 cm^{-1}. This is due to the shorter relaxation times of OH stretchings (290 ± 30 fs) compared to OD stretchings (400 ± 100 fs) [14].

Exchange of D_2O in CaOx·D_2O with atmospheric water: After the preparation of fully D_2O containing analogue of COM, the next aim was to study the displacement of D_2O in the crystal by atmospheric H_2O. It is an interesting attempt because the D_2O molecules are in the solid phase and the water molecules are in the gaseous phase The heterogeneous displacement worked out well and some water molecules entered the crystal and displaced the D_2O molecules. The experiment was carried out at the humidity of about 52%. Fig. 10 shows the K-M format of the diffuse reflectance infrared spectra of CaOx·D_2O after 1, 4 and 8 days of exposure to the atmosphere. The exchange of D_2O with atmospheric water molecules seems to be a slow process.

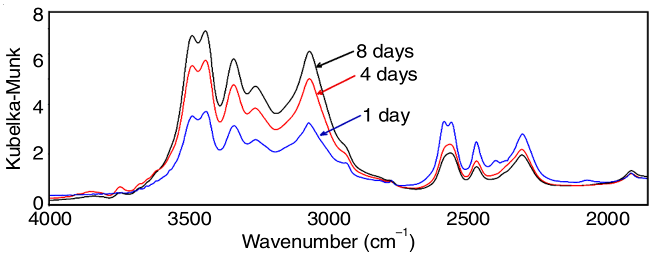

Fig. 10. Diffuse reflectance infrared spectra of calcium oxalate monodeuterium oxide after 1, 4 and 8 days exposure to the atmosphere with 50% relative humidity and 22 °C

Conclusion

It has been demonstrated in this work that a fully D_2O exchanged analogue of COM can be prepared by treating COM with D_2O in a water-free environment for 2 consecutive days. A comparison of the infrared spectra shows the OH stretchings of water molecules in the CaOx·H_2O crystal structure and OD stretchings of D_2O molecules in the CaOx·D_2O crystal structures are similar. This confirms that the D_2O molecules entering the crystal structure assume identical positions to the water molecules in CaOx·H_2O. The spectral patterns of the OH and OD stretchings in the crystal structures show that the OD stretching bands are narrower compared to OH stretching bands that are twice broader. This confirms the relaxation times of OH stretchings are shorter than the OD stretching times (Heisenberg's uncertainty principle). The analysis of the liquid mixture extracted after the exchange of crystalline water molecules with D_2O molecules reveals that about 60% of water molecules were displaced by D_2O after 6 h. The experiment performed to study the exchange of D_2O with water molecules on the fully deuterated analogue of COM with atmospheric H_2O demonstrates that the displacement in the heterogeneous phase is an extremely slow process. It took 8 days for an expected result. The preparation of a fully D_2O replaced analogue of COM paves the way to explore new studies based on the D_2O replaced analogue.

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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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