

Simplified batch equilibration for D/H determination of non-exchangeable hydrogen in solid organic material

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Hydrogen isotopic analysis of organic materials has been widely applied in studies of paleoclimate, animal migration, forensics, food and flavor authentication, and the origin and diagenesis of organic matter. Hydrogen bound to carbon (C-H) generally retains isotopic information about the water present during organic matter synthesis and associated biosynthetic fractionations, but hydrogen bound to other elements (O, S, or N) can readily exchange with atmospheric water vapor and reflects recent exposure to water or vapor. These two pools must be separated to obtain meaningful information from isotope ratios of organic materials. Previously published analytical methods either replace exchangeable H chemically or control its isotopic composition, usually by equilibration with water or waters of known isotopic composition. In addition, the fraction of H that is exchangeable can vary among samples and is itself of scientific interest. Here we report an improved and automated double-equilibration approach.

Samples are loaded in a 50-position autosampler carousel in an air-tight aluminum equilibration chamber. Water vapor of known isotopic composition is pumped through the chamber at 115°C for at least 6 h. After flushing with dry N₂ and being cooled, the carousel is rapidly transferred from the equilibration chamber to a He-purged autosampler attached to a pyrolysis elemental analyzer connected to an isotope ratio mass spectrometer. By equilibrating two aliquots of each sample with two isotopically distinct waters, it is possible to calculate both (1) the D/H ratio of non-exchangeable H, and (2) the fraction of H that is exchangeable. Relative to previous double-equilibration techniques, this approach offers significant reductions in sample size and labor by allowing simultaneous equilibration of several tens of samples. Copyright © 2009 John Wiley & Sons, Ltd.

Stable hydrogen isotope ratios (²H/¹H, or D/H) in organic materials have been used for investigations in a variety of fields, including paleoclimate,^{1–5} animal migration,^{6–8} forensics,^{9,10} and thermal maturation of organic matter.^{11,12} The lability of hydrogen atoms in organic matter can be broadly predicted based on their chemical bonding. Hydrogen that is covalently bound to non-aromatic C is largely non-exchangeable on laboratory timescales. The isotopic composition of this pool preserves information about the D/H ratio of environmental or tissue water during biochemical H fixation. In contrast, organic hydrogen bound to O, N, and S is loosely bound, 'labile' H which continuously exchanges with ambient water. The rate of exchange of labile H depends on the chemical bonding environment, pH, temperature, and steric accessibility.¹³ Labile H exchanges with atmospheric water vapor on a time scale of minutes to days, so its D/H ratio reflects the recent history of vapor exposure. The D/H ratio in atmospheric water vapor is affected by season, the movement of weather fronts, daily cycles of temperature and humidity, and even changes in building air conditioning.

Unless the effects of exchangeable H are eliminated or accounted for, the intercomparison of H results from different laboratories, or even from different days, is ambiguous.

The development of the batch equilibration technique was motivated by the need to generate precise and accurate H isotope data with greater efficiency than previous approaches. By increasing the sample throughput and decreasing the reliance on specialized technical skills, the method can be applied to a greater range of ecological, paleoclimate, and stratigraphic studies where large numbers of samples are required.

Several analytical approaches have been developed to eliminate or control the influence of exchangeable H from organic materials. For certain analytes, specific derivatization procedures can remove or replace exchangeable H (e.g. 'nitration' of cellulose, acetylation of alkanols). The contribution of any added H to the measured total H pool must be accounted for by using reagents of known H isotopic composition.¹⁴ Such approaches are effective when the chemical structure is well defined and sufficiently reactive. However, derivatization procedures are labor intensive and inappropriate for heterogeneous materials such as kerogen or bulk biomass where exchangeable H is present in many

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forms, or for degraded cellulose, which may yield low amounts of 'cellulose nitrate'.¹⁵

An alternative to removing or replacing exchangeable H is to control its isotopic composition via exchange with water in the laboratory. Methods using either liquid water^{15,16} or water vapor^{10,17–20} as the exchange medium have been demonstrated. The relationship between measured δD values of exchangeable H (δD_x), non-exchangeable H (δD_n) and measured total H (δD_t) is closely approximated by:

$$\delta D_t = x_e \delta D_x + (1 - x_e) \delta D_n \quad (1)$$

where x_e is the fraction of exchangeable H in the total H pool (see Sessions and Hayes²¹ for an exact form of Eqn. (1)). At equilibrium, the relationship between δD_x and δD_w (ambient water) is determined by the equilibrium isotopic enrichment factor ϵ_{x-w} , defined as:

$$\epsilon_{x-w} = [(1 + \delta D_x)/(1 + \delta D_w)] - 1 \quad (2)$$

In Eqn. (2) and below, we follow the recommendation of IUPAC²² to omit factors of 1000‰ from the definition of δ , i.e. $\delta D_A = (R_A/R_{std}) - 1$. Thus ϵ_{x-w} has a value $\ll 1$, but can be expressed in per mil units (‰). For any given material, the appropriate value for ϵ_{x-w} depends on temperature as well as on the structure of the molecule containing organic exchangeable H.¹⁷ While ϵ_{x-w} ranges from 30 to 110‰ for simple, pure compounds (e.g. ethanol, acetic acid, phenol),²³ values of 80 ± 20 ‰ are typical for most materials of interest to environmental scientists (proteins, cellulose, humic acids).^{17,18}

If x_e and ϵ_{x-w} can both be constrained, values of δD_n can then be determined by equilibrating samples with a single water or vapor. This approach requires a pure substance with known stoichiometry (to constrain x_e) and an experimentally established value for ϵ_{x-w} . In practice, it is possible to calculate δD_n by analyzing isotopic laboratory reference materials (with known δD_n values) having the same H exchange characteristics as the unknown samples,^{15,24} an approach known as the Principle of Identical Treatment (PIT).²⁵ This approach requires complete equilibration, and materials must have uniform and precisely known x_e and ϵ_{x-w} values. For example, when analyzing keratin, an animal protein of uniform composition and structure, Wassenaar and Hobson²⁴ demonstrated the feasibility of measuring samples and laboratory reference materials that have been exposed to the same laboratory air for 96 h. Three reference materials whose δD_n varied by up to 80‰ were first carefully calibrated using dual-equilibration techniques. Regression of the measured δD_t values of reference materials against their known δD_n values allows the calculation of the effect of water vapor on the exchangeable H pool. A correction can then be applied to the measured δD_t values of unknowns to calculate their δD_n values. Bowen and others²⁰ improved on this approach by loading samples into silver capsules which are then placed in vapor-tight chambers with a beaker of water of known isotopic composition. Samples are exposed to water vapor of controlled isotopic composition and constant relative humidity (100%). Under these experimental conditions (room temperature and a static equilibration chamber where vapor movement is controlled by diffusion), complete equilibration is achieved in 4 days.

A similar approach has been developed by Filot *et al.*¹⁹ in which tree cellulose samples are exposed to vapor of known δD_w value. A heated autosampler is configured so that its sample chamber can be purged with either dry He or H₂O vapor from a reservoir of water with known δD_w value. The purged chamber houses one sample at a time immediately prior to it being dropped into the pyrolysis elemental analyzer (EA). This system allows for precise control of vapor δD_w , but the equilibration time is limited by practical considerations to approximately 10 min, the time required for the analysis of one sample by the EA. This system has been successfully used for tree cellulose, which is typically composed of loose fibers that are highly permeable to water vapor, but longer equilibration times would be required to ensure isotopic equilibrium for other types of materials.

The rigorous requirement for uniform x_e and ϵ_{x-w} is obviously problematic for complex organic substrates such as kerogen. In these materials, x_e generally depends on variable N, S, and O content; in addition a rigid tertiary structure can render some exchangeable H sterically inaccessible to water. Moreover, the need for uniform x_e can be difficult to satisfy even for a pure, well-defined substance. For example, water vapor can easily access all hydroxyl H in amorphous cellulose, but crystalline cellulose contains some hydroxyl groups inaccessible to water vapor.²⁶ Sample preparation can affect the accessibility of water to H in hair keratin, with greater x_e observed for whole hair than in mechanically ground hair.²⁰

For samples in which the abundance of exchangeable H is unknown or variable, equilibration with two or more waters of distinct isotopic compositions offers several advantages. By analyzing aliquots of each sample after equilibration with two or more isotopically distinct waters, it is possible to solve for both x_e and δD_n simultaneously.¹⁷ For samples that have undergone diagenesis or other chemical alteration, x_e is an important metric of the degree of alteration. Earlier methods of double-equilibration required relatively large sample sizes in order to generate at least 30 μmol of H₂ gas for manual dual-inlet isotopic measurement.^{17,18} The labor-intensive manufacturing of quartz break-seal ampoules and subsequent processing of equilibrated samples on vacuum distillation lines were technically demanding and slow. In contrast, the measurement of batches of equilibrated samples using pyrolysis-elemental analysis and continuous-flow mass spectrometry yields significant savings in sample size, effort, time, and analytical expense.

EXPERIMENTAL

Materials

International reference materials polyethylene foil (IAEA-CH-7) and cellulose (IAEA-CH-3) were obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). Laboratory reference materials at Indiana University have been previously characterized using off-line techniques and include a series of *n*-alkanes (C₂₆, C₂₈, C₂₉, C₃₀, C₃₄, C₃₆, and C₃₈), 5 α -androstane, dibenzothiophene, and phenanthrene.²⁷ These materials were used to evaluate the performance of the pyrolysis elemental analyzer/isotope

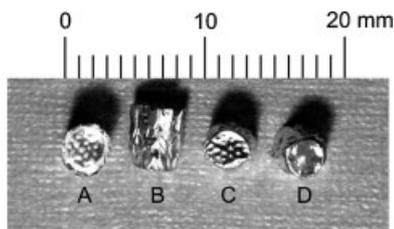


Figure 1. Silver capsules used for loading equilibrating samples. Capsules A, B, and C have been perforated to allow vapor to access sample after crimping shut. Capsule D is not perforated.

ratio mass spectrometry (pyrolysis EA-IRMS) system for memory, source linearity, and reproducibility of isotopic analyses. Deer collagen was prepared from bones found at several sites spanning isotopic gradients across the continental USA.²⁸ Powdered crab chitin (poly *N*-acetylglucosamine) was obtained from Sigma (St. Louis, MO, USA). Hydrocarbon reference materials, which contain no exchangeable H, were weighed on a Sartorius M2P microbalance to a precision of 1 μg and loaded into pre-annealed capsules made of silver foil (3.5 \times 5 mm; Costech Analytical Technologies Inc., Valencia, CA, USA), which were then crimped shut to prevent sample spillage. In order to allow vapor access to samples with exchangeable H, we used a bundle of 20 sharpened steel pins to repeatedly perforate the bottoms of stock silver capsules, resulting in \sim 60 small holes in each capsule (Fig. 1). The size of the holes was small enough to avoid spilling organic particulates. The capsules were subsequently annealed overnight at 400°C. Samples were loaded into perforated capsules and weighed. The top of each loaded capsule was folded shut using forceps.

As an alternative to puncturing of capsules with needles, Dexmet Corp. (Wallingford, CT, USA) provides precision-expanded silver foil with small openings. The silver foil can be shaped to form customized capsules or envelopes with

uniformly sized holes and increased gas permeability along all sides.²⁹ While we have not fully tested this material, it appears suitable for the application.

Collagen was extracted from several samples of deer (*Odocoileus virginianus* and *O. hemionus*) following the methods of DeNiro and Weiner.³⁰ After soft tissues had been removed, the bone was freeze-dried and ground using a mortar and pestle. Ground bone was treated with 1 N HCl for 7 days to remove minerals. The sample was rinsed to neutral pH with distilled water and freeze-dried. Lipids were removed from the organic extract using dichloromethane. The origin and preparation of kerogen from an Estonian kukersite (sample 44915) have previously been reported.³¹ The primary source of organic matter in this Ordovician shallow marine sediment is the green alga *Gloeocapsomorpha prisca*. Kerogen from the sample was isolated according to a procedure that used hydrochloric and hydrofluoric acids, organic solvents, and heavy liquids (zinc bromide solution, $\rho = 2.4 \text{ g/mL}$).³²

Coal samples were collected from a Pennsylvanian bituminous seam in the Illinois Basin, a major sedimentary basin underlying much of Illinois, Indiana and Kentucky.^{33,34} The sedimentary basin contains abundant coal deposits that are actively exploited. The coal seam is dissected by a mafic dike, and samples were collected spanning the zone of thermal alteration and into unaltered coal. Samples were collected from an active mine, ensuring minimal weathering. Kerogen was extracted from the coal in a similar method to that used for the kukersite sample.³²

Vapor equilibration

The equilibration chamber is an aluminum block (19 cm \times 19 cm \times 3 cm) that is fitted with an O-ring seal against an aluminum lid. The chamber accommodates a Costech Zero Blank autosampler carousel (Fig. 2). Two ports connect to pressure-fitted HPLC-grade Teflon[®] tubing. One port allows the introduction of dry purge gases or steam, and the other serves as a vent. We operated two equilibration chambers simultaneously to increase sample throughput. In

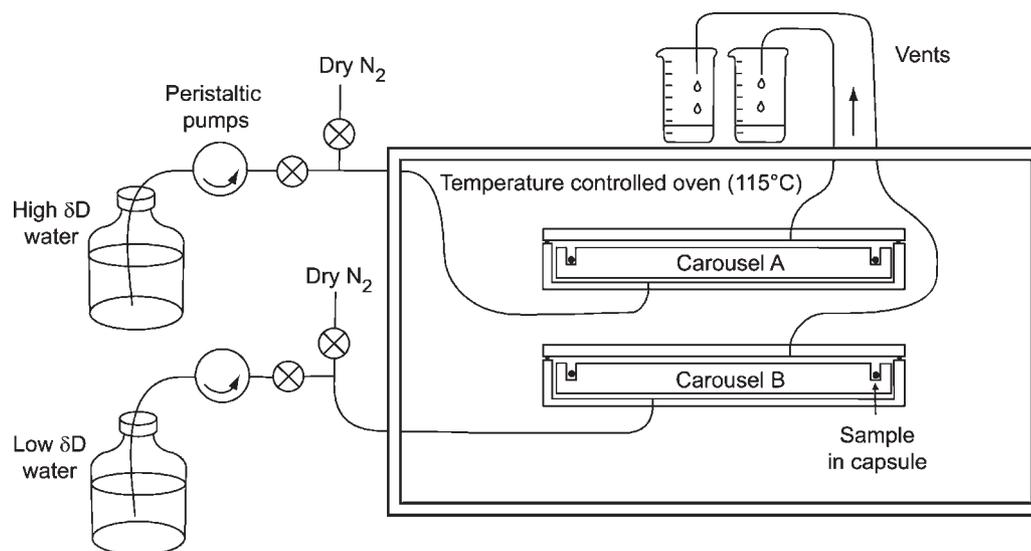


Figure 2. Schematic of water vapor equilibrator.

general, one equilibration chamber was used to equilibrate samples with D-enriched vapor while the other chamber was used for D-depleted equilibration. Both equilibration chambers fit inside a laboratory oven (interior volume: 30 cm × 30 cm × 30 cm) allowing for precise and uniform temperature control.

Samples were loaded into the carousel which was then placed into the equilibration chamber. The aluminum enclosure was clamped shut and placed in the oven, and one capillary was connected to a dry nitrogen supply. The chamber was purged for several hours with dry N₂ as the aluminum block reached the equilibration temperature of 115 ± 1°C. Water of known isotopic composition was then passed into the equilibration chamber using a peristaltic pump (Masterflex model 7553-50; Cole Parmer, Vernon Hills, IL, USA) at a flow rate of 4 mL/h. This slow flow rate ensured that all the water entering the equilibration chamber rapidly converted into steam. Steam exiting from the chamber through the second capillary tubing was condensed outside the oven in a graduated beaker to monitor the usage of water. The samples were allowed to equilibrate with steam for at least 6 h. The steam was subsequently replaced by a flow of dry N₂ gas for at least 4 h at 115°C to dry the samples. The N₂ supply was dried immediately prior to entering the equilibration enclosure by passing the gas through a coil of copper tubing immersed in liquid nitrogen. Finally, the oven was turned off and the equilibration enclosure cooled to room temperature while the flow of dry N₂ continued.

Reference materials with melting points <130°C (e.g., coumarin, *n*-alkanes) were added to the carousel at room temperature after vapor equilibration. The equilibration chamber was opened in a glove box purged with dry N₂. Capsules containing reference materials were loaded, and the equilibration chamber was again sealed. The closed chamber was then carried to the elemental analyzer, and the carousel was quickly transferred from the equilibration chamber to the autosampler. The autosampler lid was closed and the internal volume flushed with dry He to minimize exposure to atmospheric water vapor. The samples were exposed to moist laboratory air for less than 30 s during this transfer.

Isotopic analysis

The pyrolysis elemental analyzer was supplied by ThermoFinnigan (Bremen, Germany). The high temperature reactor was packed with glassy carbon granules and heated to 1410°C. The He flow rate was set to 90 mL/min. H₂ was separated from the other pyrolysis products using a ThermoFinnigan 5 Å molecular sieve gas chromatography (GC) column at 70°C. The effluent from the elemental analyzer was directed to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer which was operated in continuous-flow mode. Values of δD were calculated by comparison of sample gases with peaks of H₂ reference gas, and were anchored to the VSMOW scale by comparison with the IAEA polyethylene foil (IAEA-CH-7; δD = -100.3‰). Each sample was analyzed at least twice, after equilibrating with D-depleted water from Saskatoon, Canada (δD_w = -136‰), and with D-enriched water (δD_w = +1173‰). The isotopic compositions of both waters were established by

off-line analyses with VSMOW (Vienna Standard Mean Ocean Water) and SLAP (Standard Light Antarctic Precipitation) using reduction with uranium metal, at Indiana University,³⁵ and by laser spectroscopy at Caltech (liquid water isotope analyzer; Los Gatos Research, Mountain View, CA, USA).³⁶ Extrapolation of the VSMOW-SLAP calibration towards strongly positive δD_w values is problematic due to a paucity of reliable D-enriched reference materials. We ascertained that the δD_w value of our D-enriched water is in agreement with the estimated δD-scale for D-enriched waters (IAEA-301A, δD_w = +508.4‰, and IAEA-302B, δD_w = +996‰).³⁷ The H₃⁺ factor was measured prior to each run and was generally between 4.4 and 6.2 ppm/nA over the 10 months during which these experiments were conducted. The ideal sample size produced peaks with heights of 6–10 nA. The target sample weights depended on H content, ranging from 0.2 mg for IAEA-PEF and pure hydrocarbons to ~1 mg for kerogens.

RESULTS

In order to test the reproducibility of vapor equilibration and the effects of sample heterogeneity on δD_n and x_e, multiple aliquots of deer collagen (JTNP1) and tree cellulose (IAEA-C3) were analyzed (Table 1). Mean δD_t values for the collagen equilibrated with D-depleted (δD_w = -136‰) and D-enriched (δD_w = +1173‰) waters were -49.8 ± 1.4‰ (n = 13) and +223 ± 2.1‰ (n = 13), respectively. By assuming ε_{x-w} = 80‰, Eqns. (1) and (2) can be solved for x_e and δD_n simultaneously. The low variance of x_e (19.3 ± 0.2%) and δD_n (-45.4 ± 1.7‰) indicates that the collagen sample is well homogenized and that vapor equilibration is effective and reproducible. Analysis of IAEA-C3 cellulose (Table 1) indicates uniform exchangeability of 20.5 ± 0.1% and δD_n = -35.5 ± 2.1‰.

The effect of perforations on the rate of vapor equilibration was measured by loading aliquots of JTNP1 collagen into perforated and unperforated silver capsules. After equilibration with D-enriched (+1173‰) water for 6 h, the measured values of δD_t of collagen were 14‰ higher for samples in perforated capsules than for those in unperforated capsules. Values of δD_t for samples equilibrated with D-depleted (-136‰) water were identical for both types of capsules. The calculated x_e values increased by 0.7% when using perforated capsules.

To evaluate the equilibration technique on more complex organic samples expressing a larger range of x_e, we analyzed two sequences of kerogens prepared from a bituminous coal seam that had been intruded by magmatic dikes.^{38,39} In addition, we re-analyzed several samples that had been previously measured via the approach of Schimmelmann¹⁷ in which vapor equilibration is performed in flow-through quartz ampoules. After equilibration with water vapor and the water vapor being removed by flushing with dry nitrogen, the tubes were evacuated and sealed using an oxy-gas torch. The ampoules were combusted to yield CO₂ and H₂O. The water was cryogenically isolated and converted into H₂ over hot uranium. The hydrogen isotope ratios were measured by dual-inlet isotope ratio mass spectrometry (IRMS) and the results are presented in

Table 1. Analyses of powdered deer collagen and IAEA-CH3 cellulose. δD_t values of samples are determined by pyrolysis EA after equilibrating with either Saskatoon water ($\delta D_w = -136\text{‰}$) or D-enriched water ($+1173\text{‰}$). x_e and δD_n are calculated using Eqns. (1) and (2) assuming that $\epsilon_{x-w} = 80\text{‰}$. x_e is the fraction of hydrogen (in % of total hydrogen) that is exchangeable. δD_n is the hydrogen isotopic composition of the non-exchangeable organic hydrogen

Sample	δD_t after equilibration with low δD water (‰)	δD_t of sample equilibrated with high δD water (‰)	x_e (%)	δD_n (‰)
Deer collagen (JTNP1)	-49.6	223.6	19.3	-45.2
	-51.1	227.0	19.7	-47.0
	-50.8	220.6	19.2	-46.8
	-49.4	220.4	19.1	-45.1
	-51.0	224.0	19.4	-46.9
	-51.0	223.0	19.4	-46.9
	-47.9	222.0	19.1	-43.1
	-49.7	226.6	19.5	-45.3
	-47.5	225.3	19.3	-42.6
	-47.6	221.9	19.1	-42.8
	-51.0	224.2	19.5	-46.9
	-49.2	221.9	19.2	-44.7
	-51.0	221.8	19.3	-47.0
	Average $\pm 1\text{-}\sigma$ standard deviation	-49.8 ± 1.4	223.3 ± 2.1	19.3 ± 0.2
IAEA C3 cellulose	-41.4	247.3	20.4	-34.5
	-41.0	248.6	20.5	-34.1
	-42.5	247.2	20.5	-35.9
	-45.8	247.5	20.7	-40.0
	-42.3	246.6	20.4	-35.7
	-42.8	246.0	20.4	-36.3
	-40.9	247.5	20.4	-34.0
	-40.3	246.1	20.2	-33.2
Average $\pm 1\text{-}\sigma$ standard deviation	-42.1 ± 1.7	247.1 ± 0.9	20.5 ± 0.2	-35.5 ± 2.1

Table 2. The δD_n values obtained using the equilibration chamber and by pyrolysis EA compare favorably with those using flow-through quartz ampoules. The x_e values obtained by each method agree broadly, although the calculated x_e values are higher for three of the four materials analyzed (i.e. kerogen, chitin, and cellulose) when quartz-tube equilibration is employed.

DISCUSSION

Relative to the traditional approach of quartz-tube equilibration,¹⁷ our new batch equilibration technique achieves greatly increased efficiency in terms of sample throughput. The quartz tube equilibration technique requires considerable glass-blowing skill to form the break-seal ampoules, and requires access to vacuum distillation lines equipped with a custom-made Toepler pump. A skilled operator can process only a few (8–10) samples per day. In contrast, the batch

equilibrator allows the simultaneous processing of up to 49 samples in the same chamber. By using a commercially available pyrolysis EA fitted with an autosampler as the inlet to the IRMS instrument, approximately 49 samples per day can be analyzed.

The analytical precision estimated from replicate analyses of collagen and cellulose was $\pm 0.9\text{--}2.1\text{‰}$ ($1\text{-}\sigma$), comparable with the precision that we typically obtain for analytes with no exchangeable hydrogen. Thus, the automated equilibration procedure does not appreciably reduce the attainable level of precision. The values for the fraction of exchangeable H (x_e) and δD_n are precisely determined, with x_e reproducible within $\pm 0.2\%$, and δD_n within $\pm 2.1\text{‰}$.

Because of the paucity of reference materials that both contain exchangeable H and have known δD_n values, the accuracy of the new method was assessed by analyzing four materials using both the quartz-tube equilibration and the batch equilibration techniques (Table 2). The materials

Table 2. Reference materials equilibrated and isotopically measured using pyrolysis EA and off-line. Isotopic data are presented in ‰ vs. VSMOW. x_e is the fraction of hydrogen (in % of total hydrogen) that is exchangeable. δD_n is the hydrogen isotopic composition of the non-exchangeable organic hydrogen

Sample	Equilibration chamber and pyrolysis EA		Equilibration and close-tube combustion	
	x_e (%)	δD_n (‰)	x_e (%)	δD_n (‰)
Cellulose (IAEA-C3)	20.5 ± 0.2	-35.5 ± 2.1	34.1 ± 1.4	-30.0 ± 1.5
Kukersite kerogen	3.9 ± 0.4	-104.7 ± 2.0	6.9 ± 0.3	-105.2 ± 2.8
Chitin	13.7 ± 0.2	-40.8 ± 1.0	19.4 ± 0.2	-54.3 ± 1.5
Deer collagen	21.3 ± 0.4	-59.6 ± 2.3	22.4 ± 0.2	-65.9 ± 2.8

(collagen, chitin, kerogen, and cellulose) represent organic matter derived from a range of animal, plant, and geological sources. Values of x_e derived from both equilibration methods are highly correlated, but the x_e estimates are approximately 50% higher when quartz tubes are used for three of the four materials analyzed (cellulose, kukersite kerogen, and chitin). Apparently, vapor in quartz tubes more efficiently accesses some of the sterically hindered exchangeable H. The observed values of x_e for cellulose, chitin, and kerogen exhibit the same ratio (approximately 6:3:1) when either equilibration technique is used. However, the two equilibration techniques produce identical x_e values for collagen (21.3% and 22.4%). Further, using a static vapor equilibrator,¹⁸ Leyden and others³ reported a similar x_e for collagen from modern bison of $20.0 \pm 2.5\%$, and Cormie and others⁴⁰ measured the x_e of collagen at 20–23%. Similarly, Filot *et al.*¹⁹ reported x_e values for cellulose of 23.6%, relatively close to the value obtained by our batch-equilibration experiment (20.5%) and significantly lower than the 30% implied by stoichiometric consideration of a hexose polymer. It appears that in some materials (kerogen, chitin, and cellulose), 'exchangeable' H occupies a range of positions of varying accessibility by vapor, and the value observed for x_e depends on the equilibration technique. The patterns of x_e variability are expected to be similar for any of these materials when a single analytical method is used. Thus, the observed x_e trends in metamorphosed dikes illustrated in Fig. 3 would be similar when analyzed using different techniques, although the curve might move up or down on the x_e axis.

In contrast, values of x_e for collagen are identical when four different analytical approaches are used, suggesting that virtually all of the exchangeable H is easily accessible to water vapor. Collagen found in bones is not a structural tissue but rather serves to facilitate crystallization of mineral constituents of bone. As such, collagen fibers may be more finely arranged than fibers of tree cellulose or chitin, which are structural tissues and gain strength by closely packing polymer chains. This would account for a smaller proportion of sterically hindered exchangeable H in bone collagen.

Values of δD_n determined using the two methods match well for the cellulose, kerogen, and collagen samples, agreeing within 6‰ (somewhat larger than 2- σ analytical uncertainty). This discrepancy is small in relation to the range of δD_n found in most biological and geological systems, so the batch-equilibration technique can be applied to typical hydrogen isotope studies. However, the two equilibration methods produce different δD_n values for chitin (−40.8‰ when analyzed in the batch equilibrator, −54.3‰ when equilibrated in quartz tubes). The 13.5‰ difference exceeds the 2- σ uncertainty estimates. While this difference may result from heterogeneity in the coarsely powdered chitin, it suggests that certain complex polymers may yield different results depending on the choice of analytical method.

Because the δD value of C-bound H often differs from that of O,N,S-bound H, any inefficiency in the exchange of labile H due to steric hindrance will cause bias in the calculated δD_n . When analyzing materials with complex structures or chemical heterogeneity, organic H cannot simply be classified into two groups (exchangeable vs. non-

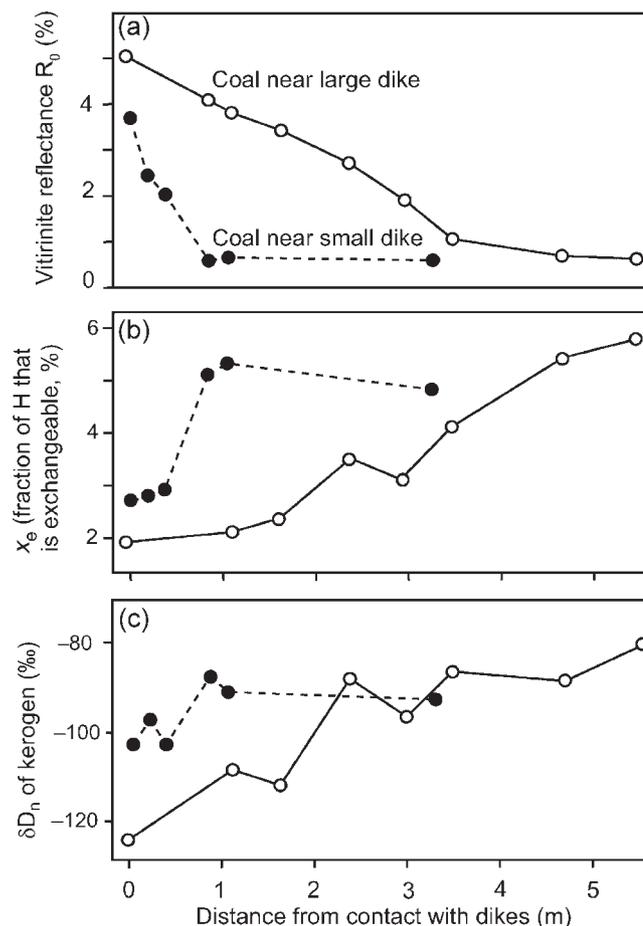


Figure 3. Effects of thermal alteration of coal kerogen near igneous dikes. (a) Vitrinite reflectance increases along thermal alteration, whereas (b) the fraction x_e of exchangeable organic hydrogen (in % of total hydrogen) and (c) the δD_n value of non-exchangeable hydrogen in kerogen decrease due to thermal elimination of chemical functional groups.³⁹ Open circles indicate data from coal near the large dike, whereas black circles refer to the coal near the small dike.

exchangeable), but rather covers a spectrum of intermediate exchangeability. The bias in measured δD_n is minimized by subjecting all samples to the same equilibration conditions. Moreover, by routinely measuring x_e for all samples, potential problems resulting from inconsistent equilibration, incomplete drying, and sample heterogeneity can be spotted.

The value of determining both x_e and δD_n in an investigation of organic matter is illustrated by the results for the coal kerogen subjected to contact metamorphism near dikes, shown in Fig. 3. Heating of sedimentary organic matter causes a variety of changes in chemical properties including dehydration reactions, elimination of functional groups containing N, S and O, and cleavage of ether- and ester-linked carbon chains. The two dikes vary in thickness and provide an opportunity to test the effects of variable heating under natural conditions. Near each dike, maximum temperatures of 330 to 350°C during contact metamorphism caused changes in several chemical and physical properties including reduction of organic H and N, and an increase in vitrinite reflectance. Outside the contact aureole, x_e ranges from 5 to 6%, but is reduced to 2.0 to 2.5% at the contact with

the dike due to thermal elimination of functional groups containing exchangeable H. Outside the contact aureole, kerogen δD_n values range from -80 to -90‰ , and decrease to -103‰ near the small dike and -120‰ near the large dike. The decrease in δD_n thus testifies to the thermal elimination of relatively D-enriched functional groups from coal kerogen during contact metamorphism.³⁹

In complex organic materials, it is impossible to divide all H into two distinct pools of exchangeable and non-exchangeable H. Different pools of H equilibrate at different rates, which vary as a function of temperature and vapor pressure, among other factors. Here, we use the operational definition that H is exchangeable if it equilibrates with water vapor at 115°C within 6 h. Experiments equilibrating IAEA-CH-3 cellulose and JTNP1 collagen for 4, 6 and 10 h produced identical δD_n and x_e values showing that apparent equilibrium has been reached in the equilibration times used in this study. However, differences in the sample preservation, crystalline structure, and pretreatment (grinding vs. whole) can affect x_e for relatively pure samples such as hair and tree cellulose.^{20,26} Moreover, in complex organic materials such as kerogen, H may occupy a wide range of positions in the polymerized and cross-linked matrix, thereby occupying a greater continuum between easily exchangeable positions and non-exchangeable positions. While the equilibration conditions used in this study (115°C for 6 h) are sufficient for isotopic exchange in cellulose and collagen, additional tests of the equilibration efficiency are required for studies of more complex materials.

This batch vapor equilibrator can provide reliable δD_n and x_e values for materials containing exchangeable H provided that the materials are sufficiently homogeneous, permeable to vapor, and have melting points above the chosen equilibration temperature. Hygroscopic materials such as simple sugars and organic acids pose analytical challenges because of difficulties in completely removing adsorbed water by flushing with dry N_2 . Extreme care must be taken to keep atmospheric water vapor from accessing samples after equilibration prior to analysis by pyrolysis EA-IRMS.

CONCLUSIONS

We have developed an automated procedure for the simultaneous equilibration of bulk organic samples with water vapors of two different isotopic compositions prior to analyses by pyrolysis EA-IRMS. This procedure greatly simplifies the hydrogen isotopic analysis of organic materials containing exchangeable hydrogen. A complete carousel can be equilibrated simultaneously with the same water vapor. Although we used 50-position carousels in this study, commercially available 100-position carousels (Costech Analytical Technologies) could further increase sample throughput. By using two parallel chambers and water reservoirs, it is possible to double-equilibrate separate aliquots of each sample and analyze by pyrolysis EA-IRMS, allowing the determination of both the δD_n of non-exchangeable H and the x_e of over 40 samples within a 24-h period. Equilibration times of 6 h at 115°C generally achieve complete equilibration for the cellulose, kerogens, and proteins examined here. However, equilibration times and

temperatures can be altered for materials with low melting points or with lower exchange rates. Once the vapor equilibration is finished, complete removal of water vapor and any adsorbed water is essential. The carousel must be transferred from the equilibration chamber to the He-purged autosampler as quickly as possible to minimize exposure to atmospheric moisture.

The batch equilibrator described here offers several advantages over previous equilibration techniques. By equilibrating entire carousels with the same water vapor, the per-sample effort is greatly reduced relative to previous off-line techniques. The temperature and duration of equilibration are flexible. With a simple means of equilibrating samples with two distinct water vapors, the 'dual-equilibration' approach for determining both δD_n and x_e can be used as a standard laboratory procedure, eliminating the uncertainties of equilibrating with a single water. Routine employment of dual-vapor equilibration will facilitate the comparison of δD_n analyses performed by different laboratories.

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