

# Determination of the H<sub>3</sub> Factor in Hydrogen Isotope Ratio Monitoring Mass Spectrometry

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The H<sub>3</sub> factor,  $K$ , is a parameter required in high-precision, mass spectrometric analyses of hydrogen isotopic abundances. When H<sub>2</sub> is used as the sample gas,  $R^* = R - Ki_2$ , where  $R^*$  is the true HD/H<sub>2</sub> ratio,  $R$  is the observed (mass 3)/(mass 2) ion-current ratio, and  $i_2$  is the ion current at mass 2. Four different methods for the determination of  $K$  were defined and tested under conditions characteristic of isotope ratio monitoring systems. Three of these were peak-based. The fourth employed steady flows of H<sub>2</sub> from a conventional inlet system. Results obtained using the latter method were more precise (standard deviation of  $K = 0.1$  versus  $\sim 0.6$  ppm mV<sup>-1</sup> for the peak-based methods). However, use of the resulting values of  $K$  for correction of isotope ratio monitoring GC/MS results led to systematic errors as large as 9‰, whereas use of the peak-based values led to no systematic errors. Values of  $K$  were only weakly dependent on the pressure of He, declining  $\sim 5\%$  for each 10-fold increase in  $P_{\text{He}}$ . Small variations in partial pressures of H<sub>2</sub>O and CH<sub>4</sub>, potential contaminants under isotope ratio monitoring conditions, had no significant effect on values of  $K$ .

The abundance of <sup>2</sup>H (D) is low relative to that of <sup>1</sup>H (H): at natural abundance, most organic materials contain less than 100 ppm D. To avoid confounding contributions from other, more abundant heavy isotopes (e.g., <sup>13</sup>C in CH<sub>4</sub>), highly precise measurements of hydrogen isotopic ratios typically employ molecular H<sub>2</sub> as the sample gas for mass spectrometric determination of H<sub>2</sub> and HD abundances. An unfortunate consequence of the use of H<sub>2</sub> for these measurements is that the reaction



occurs readily in the ion source of the mass spectrometer.<sup>1</sup> Since H<sub>3</sub><sup>+</sup> is not resolved from HD<sup>+</sup> by typical isotope ratio mass spectrometers, a correction is required. In materials containing a

natural abundance of D, H<sub>3</sub><sup>+</sup> can account for as much as 5–30% of the  $m/z$  3 signal, so the correction for H<sub>3</sub><sup>+</sup> is significant.

In “conventional” isotope ratio measurements, both sample and standard gases enter the ion source as pure H<sub>2</sub> via dual, viscous-leak capillaries. Ion source pressures are typically 10<sup>-7</sup> mbar or lower during these measurements, and H<sub>2</sub> is the only neutral species present in significant quantities. Under these conditions, reaction 1 is the only important source for H<sub>3</sub><sup>+</sup>,<sup>1</sup> so that the production of H<sub>3</sub><sup>+</sup> is given by

$$[\text{H}_3^+] \propto [\text{H}_2^+][\text{H}_2] = K[\text{H}_2]^2 \quad (2)$$

The proportionality constant in this equation ( $K$ ) is commonly known as the “H<sub>3</sub> factor”, and for dual-inlet measurements, it is both readily measured and reliably stable. In practice, the value of  $K$  is usually determined by measuring the (mass-3)/(mass-2) ion current ratio of a sample over a range of pressures and using least-squares regression to find the value of  $K$  (i.e., the slope) in the expression

$$R = \frac{i_3}{i_2} = \frac{(i_{\text{HD}} + i_{\text{H}_3})}{i_{\text{H}_2}} = \frac{i_{\text{HD}}}{i_{\text{H}_2}} + Ki_{\text{H}_2} = R^* + Ki_{\text{H}_2} \quad (3)$$

where  $R$  is the measured ion current ratio,  $R^*$  is the corrected HD/H<sub>2</sub> ratio,  $i_2$  and  $i_3$  are the ion currents measured at mass-2 and -3, and  $i_{\text{H}_2}$ ,  $i_{\text{HD}}$ , and  $i_{\text{H}_3}$  are the ion currents due solely to H<sub>2</sub><sup>+</sup>, HD<sup>+</sup>, and H<sub>3</sub><sup>+</sup> ions, respectively. Once the value of  $K$  is known, eq 3 serves as the basis for correction of subsequent dual-inlet analyses.<sup>1</sup> In modern isotope ratio mass spectrometers,  $K$  typically has a value of 5–30 ppm mV<sup>-1</sup> (equivalent to 5–30‰ nA<sup>-1</sup>) and is stable to within  $\sim 1\%$  relative over a period of many hours. D/H ratios can accordingly be determined with a precision of 1‰ or better.

In contrast, recently developed methods utilize isotope ratio monitoring mass spectrometry (irmMS) for the measurement of D/H ratios.<sup>2–6</sup> In these analyses, H<sub>2</sub> is carried to the ion source in a stream of carrier gas (typically He), and D/H ratios are

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obtained by the integration of mass-2 and -3 ion currents across sample peaks. The fundamental differences between isotope ratio monitoring and conventional, dual-inlet analyses are (i) the dynamic conversion of samples into H<sub>2</sub>, (ii) the presence of He carrier gas, and (iii) measurement across large variations in the partial pressure of H<sub>2</sub>. All of these factors potentially lead to changes in both the mechanism and magnitude of H<sub>3</sub><sup>+</sup> formation.

In a companion paper (Sessions et al., preceding paper), we discuss the steps necessary to correct isotope ratio monitoring data for H<sub>3</sub><sup>+</sup> and show that a form of the pointwise correction first described by Tobias et al.<sup>7</sup> is the most broadly applicable. Conceptually, the pointwise correction is very similar to the approach used for dual-inlet analyses, and it depends critically on two of the same requirements: that *K* can be easily measured and that it is reliably stable. In this report, we examine those requirements under typical isotope ratio monitoring conditions, show that the value of *K* is remarkably stable under widely varying ion source conditions, and evaluate several alternative methods that can be used to determine the value of *K*.

## EXPERIMENTAL SECTION

**Mass Spectrometry.** Hydrogen isotope ratio monitoring measurements were made using a Finnigan-MAT 252 isotope ratio mass spectrometer (IRMS) which was modified in our laboratory. The system consists of a Varian 3400 gas chromatograph (GC) equipped with a cool on-column injector, coupled to the IRMS via a pyrolysis reactor and conventional open split. Operation of the pyrolysis reactor, which quantitatively converts organic H to H<sub>2</sub>, was described by Burgoyne and Hayes.<sup>8</sup> Currently, we use a 3.18-mm-o.d. by 0.50-mm-i.d. alumina tube (99.8% purity, Vesuvius-McDanel, Beaver Falls, PA) to conduct the gas through a ~15-cm zone heated to 1400 °C. No water-removal device is employed in our system. Pyrolysis over graphite reduces background water signals to <2 pA, better than can be achieved with drying systems based on Nafion membranes.<sup>9</sup>

Approximately 0.2 mL/min of the analyte gas stream is introduced to the IRMS via an open split. Gas flowing to the open split is controlled in conjunction with a vent installed upstream from the pyrolysis reactor so that the solvent peak from GC injections can be diverted from the reactor in a manner analogous to that used for carbon irmGC/MS.<sup>10</sup> While the pyrolysis reactor is not damaged by introduction of the solvent peak, the resulting accumulation of tarlike pyrolysis products downstream from the reactor adversely affects peak shape in subsequent samples.

The MAT-252 was modified to prevent scattered or low-energy <sup>4</sup>He<sup>+</sup> ions from reaching the mass-3 collector by placing an electrostatic lens after the mass-3 slit, just before the mass-3 Faraday detector. The lens consists of two plates, 8.00 mm long (along the beam path) and 4.34 mm apart. When the IRMS is operating at 6.6 kV accelerating potential, the lens is held at ~5.3 kV, thus providing a potential energy barrier which repels ions

with less than 80% of nominal energy. The voltage on each plate is controlled independently to facilitate steering of the ion beam after the slit, which is necessary to accommodate the limited space available for the detector. Two shields are placed between the lens and the Faraday cup and are held at ground potential and -95 V, respectively. These shields help prevent leakage of secondary electrons from the Faraday cup, a process that would otherwise be stimulated by the strong electric field of the energy-filtering system. The system described here reduces the ion current reaching the mass-3 detector to <60 fA with 200 μL/min He flowing into the IRMS (the maximum flow rate the MAT-252 can accept). In the absence of the energy filter, the scattered ion current reaching the mass-3 collector is >10 pA. This system is similar in its operation and performance to that described by Hilker et al.<sup>4</sup> for the Finnigan Delta+XL mass spectrometer.

Isotope ratio monitoring data were processed using Visual Basic codes which we have developed for use in Excel (Microsoft, Redmond, WA). These use the pointwise correction algorithm described by Sessions et al. (preceding paper). Data were collected using digitization intervals of 250 (for *n*-alkane analyses) or 125 ms (for all other experiments). Except where noted, the time constant of the mass-2 amplifier was 200 ms.

**Determination of *K*.** We evaluated four separate methods for determining the value of *K* for use in isotope ratio monitoring systems. These were as follows:

(1) "*Bellows Method*". This is the method commonly used with dual-inlet analyses, in which pressure of an H<sub>2</sub> test gas in the sample reservoir is adjusted sequentially to yield different signal levels. In the present work, helium was supplied via the GC interface to maintain the ion source pressure at levels typical of isotope ratio monitoring (~10<sup>-5</sup> mbar indicated pressure). Ion currents were integrated for 5 s at each signal level and linear regression of the measured ion current ratio on the mass-2 ion current yielded the value of *K* via eq 3. This method was used both under computer control (using the Isodat software) and manually. In the latter case, data were collected in isotope ratio monitoring mode and signals were integrated using Excel. The only significant difference between computer- and manually controlled measurements was that the version of Isodat software employed here (v7.2-00) does not correct data for background signals prior to calculating the regression, whereas background signals were removed during integration in Excel.

(2) "*Peak-rms Method*". The *n*-alkane standard described by Sessions et al. (preceding paper), in which concentrations of the 15 *n*-alkanes vary by 5-fold, was first analyzed using the irmGC/MS system. Then multiple sets of δD values were calculated using a range of values of *K*, keeping all other parameters constant. The best value of *K* was taken as that which produced the smallest root-mean-square (rms) error.

(3) "*Peak-Slope Method*". Using the same data collected for method 2, the value of *K* was chosen as that which produced no correlation between peak height and the error in δD. That is, *K* was varied until a linear regression of δD error on peak height produced a slope of zero. Methods 2 and 3 are effective in determining *K* only because of the very large concentration differences in the sample and because the mixture of standard alkanes was designed with no correlation between δD and the concentrations of the analytes, thus allowing separation of mass-

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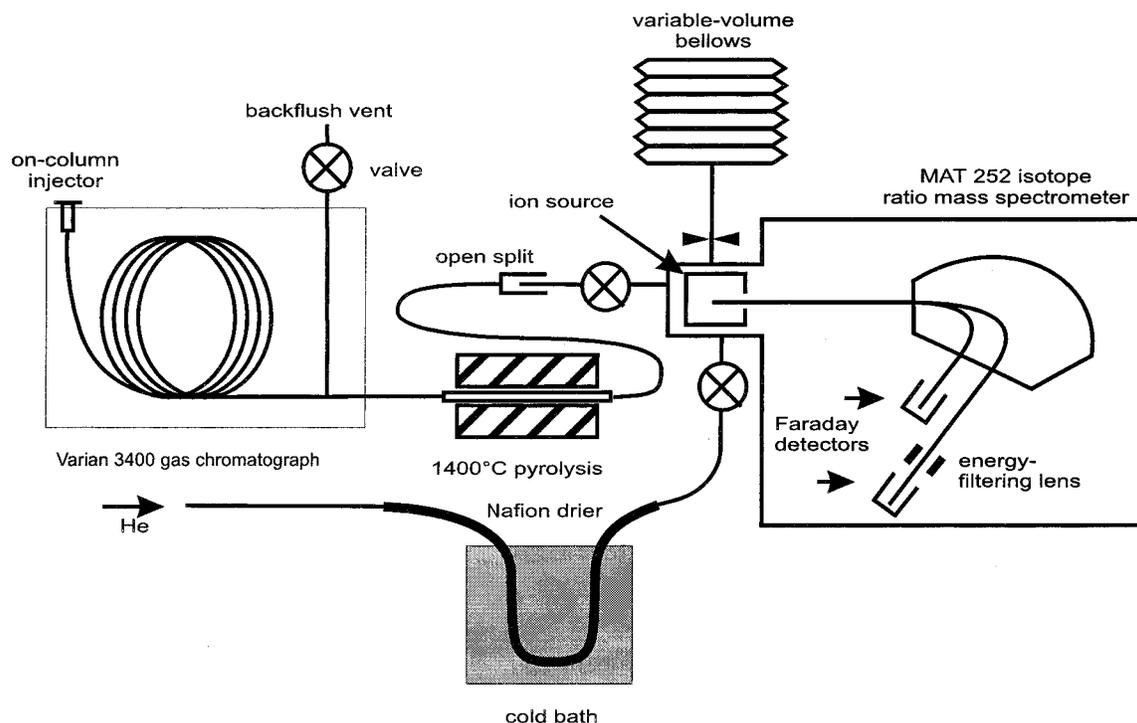


Figure 1. Diagram of the hydrogen isotope ratio monitoring analytical system. Operation of the various components is described in the text.

dependent (i.e., ion current ratio) effects from peak height-dependent  $H_3^+$  effects.

(4) "Instantaneous Method". The electronic time constant of the mass-2 amplifier was adjusted to carefully match that of the mass-3 amplifier at 1.30 s. Broad peaks of varying size were produced by slowly injecting a mixture of  $CH_4$  and He into the GC using a syringe. The resulting hydrogen peaks were integrated at 62-ms intervals and  $K$  was calculated by regression of  $R$  versus  $i_2$  for the individual data points comprising the peak. This method was tested in part to examine whether  $K$  is truly constant during rapid changes in  $P_{H_2}$ . For example, rapid changes in space-charge distribution within the ion source of the IRMS during the introduction of an  $H_2$  peak could lead to varying rates of  $H_3^+$  formation which do not follow eq 2.

**Variable Ion Source Conditions.** To investigate the magnitude of changes in  $K$  that typical isotope ratio monitoring conditions might produce, we systematically measured  $K$  with changing ion source pressure ( $\sim P_{He}$ ), under changing partial pressures of  $H_2O$  and  $CH_4$ , and with changing background concentrations of GC column bleed. These compounds were chosen for investigation because (i) He is abundant during isotope ratio monitoring analyses, (ii)  $H_2O$  and  $CH_4$  are likely to participate in proton-exchange reactions,<sup>11,12</sup> and (iii) varying column bleed contributions represent the largest change that occurs over the course of a typical GC analysis. Although the partial pressure of He is generally constant throughout isotope ratio monitoring experiments, small fluctuations may arise when, for example, solvent peaks are back-flushed, external standards are injected, etc. In all examinations of such ion-source effects, values for  $K$  were calculated using the bellows method (see above).

Partial pressures of He and  $CH_4$  were varied by means of pressure regulators and capillary leaks. For the He experiments, relatively high flow rates were controlled by the addition of a needle valve after the capillary leak. The  $H_2$  was introduced using a separate leak and did not flow through the needle valve. Pressures of He in the ion source were calculated from gauge readouts using an estimated ion source conductance of 18 L/s (for  $^4He$ ), a pumping speed of 50 L/s at the Penning gauge, and a 6.8-fold decrease in sensitivity to He (relative to  $N_2$ , the calibration gas) by the Penning gauge. On this basis, pressures of He in the ion source are typically  $10^{-4}$  mbar (gauge readings of  $\sim 10^{-5}$  mbar) during isotope ratio monitoring analyses. In this experiment, they were varied from  $10^{-7}$  to  $10^{-4}$  mbar. The partial pressure of  $CH_4$  was measured in terms of the  $m/z$  16 ion beam current, and varied from 0.1 to 11 nA. For comparison, the background  $CH_4$  ion current during actual irmGCMS analyses is not detectable above the  $\sim 0.3$  pA background current at  $m/z$  16, which is presumably due to  $^{16}O^+$ . GC column bleed was varied by changing the temperature of the GC and allowing  $\sim 5$  min for the column bleed to stabilize at each temperature. The amount of column bleed at each temperature was quantified using the  $m/z$  2 ion beam current, which varied from  $\sim 0$  at a column temperature of 40 °C to 0.6 nA at 325 °C.

Changes in  $K$  with the partial pressure of  $H_2O$  in the ion source were studied by addition of a Nafion membrane humidistat to the system.<sup>9</sup> The arrangement is shown in Figure 1. Helium carrier gas, which had been dried by passing through the pyrolysis reactor and which had a partial pressure of water corresponding to an  $m/z$  18 ion current of  $\sim 1$  pA, flowed through a Nafion tube immersed in a variable-temperature cold bath. The outside of the Nafion membrane was purged with He directly from a gas cylinder. The concentration of  $H_2O$  in this gas was higher than that in the pyrolyzed carrier gas, so the Nafion membrane served

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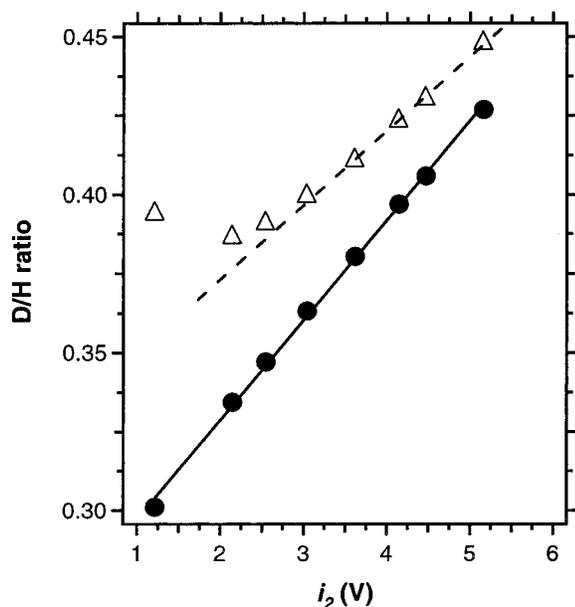


Figure 2. Typical “H<sub>3</sub> plot” used to calculate the value of  $K$ . Uncorrected data (triangles) are nonlinear because of mixing between the sample gas and background signals. After correcting for background ion currents, a linear relationship is obtained (circles). Note that the use of uncorrected data to determine the value of  $K$  will result in erroneously low values (dashed line with lower slope).

to add H<sub>2</sub>O to the carrier gas stream. Changing the temperature of the cold bath over the range  $-40$  to  $+30$  °C resulted in H<sub>2</sub>O ion currents ranging from 10 to 600 pA.<sup>9</sup>

## RESULTS AND DISCUSSION

The methods used for measuring the magnitude of  $K$  embody three general approaches. The bellows method employs measurements of constant H<sub>2</sub> signals. The peak-rms and peak-slope methods compare multiple peaks of different sizes, and the instantaneous method uses dynamic measurements of a rapidly changing H<sub>2</sub> signal. Each approach has strengths and weaknesses, and no single approach appears to be the best for all situations.

**Static Measurements of  $K$ .** Two problems affect methods based on measurements of constant H<sub>2</sub> signals. First, substantial mass-2 and -3 background currents are present under isotope ratio monitoring conditions. These potentially result both from He (as <sup>4</sup>He<sup>2+</sup> and <sup>3</sup>He<sup>+</sup> ions, or from <sup>4</sup>He<sup>+</sup> scattered into the mass-2 collector, which is not protected by an energy-filtering lens) and from hydrogen in the carrier gas. Significant background H<sub>2</sub> signals can be generated, for example, by the pyrolysis of H<sub>2</sub>O in the carrier gas. With background currents present, the relationship between  $R$  and  $i_2$  will be nonlinear (Figure 2) if an appreciable portion of the background is due to He and/or if the isotopic composition of the hydrogenic background differs significantly from that of the sample. For these reasons, measurements using the bellows method under the control of Isodat (which did not correct for background signals) produced values of  $K$  that varied systematically with the range of H<sub>2</sub> signals employed, and that were up to 10% smaller than those determined with background correction.

In our system, background currents due to H are small enough that they can be neglected, and the entire background (due therefore to He) can simply be subtracted to give a linear

relationship (Figure 2). Note that if the H background were significant, this analysis would become considerably more difficult because the H background—which would contribute to formation of H<sub>3</sub><sup>+</sup>—cannot simply be subtracted along with the He background (see Sessions et al., preceding paper). Some value of  $K$  would have to be assumed to correct for the H<sub>2</sub> background, with the true value of  $K$  then being found through successive approximations.

In principle, these difficulties can be overcome by subtracting the portion of the background signal due to He and by minimizing the H portion of the background. In practice, however, distinguishing the contributions of He and H to the background can be challenging. By examining the background signals in a He carrier gas that was passed sequentially through CuO at 800 °C (to combust H<sub>2</sub> to H<sub>2</sub>O) and then through liquid nitrogen (to trap H<sub>2</sub>O), we were able to estimate the relative contribution of H to the background signals in our instrument. This process is tedious, however, and would not be suitable for use on a frequent basis. An alternate possibility, if background signals are not too large, is to measure the value of  $K$  at very large values of  $i_2$ , where the effects of background signals are minimized.

The second problem facing measurements based on constant ion currents is that these measurements do not fully duplicate the conditions encountered during isotope ratio monitoring experiments. Specifically, the rapid changes in  $P_{H_2}$  that occur as sample peaks pass through the ion source are not reproduced. Also, trace levels of gases such as H<sub>2</sub>O, CH<sub>4</sub>, CO, and N<sub>2</sub> may increase throughout the course of an analysis as column bleed and atmospheric leaks increase in the GC. The accuracy and applicability of a static measurement of  $K$  to isotope ratio monitoring conditions are therefore uncertain.

**Peak-Based Measurements of  $K$ .** The peak-based measurements provide an ideal basis for comparison with the static measurements because they are derived by completely independent means. The large variations in peak height in our alkane test mixture allow use of both the peak-rms and peak-slope methods to determine values of  $K$ . In the peak-rms method, the relationship between the rms error of the  $n$ -alkane standard and the value of  $K$  was always a smooth, parabolic curve (Figure 3). The peak-slope method produced a nearly linear relationship when the slope of the regression was plotted as a function of  $K$ .

To determine whether these different methods produce equivalent results, we measured  $K$  using the bellows, peak-rms, and peak-slope methods over two periods of time, as follows. Typically, each morning before any samples were run, one or two aliquots of the  $n$ -alkane standard were analyzed, followed by measurement of  $K$  using the bellows method, followed by one or two additional injections of the  $n$ -alkane standard. Observations were made over two separate time periods:  $\sim 65$  days during January–March 1999 and  $\sim 30$  days during January 2000. In all, values of  $K$  were accumulated from 27 bellows measurements and 105 alkane measurements.

Values of  $K$  determined by the peak-rms and peak-slope methods are compared in Figure 4a. The two methods give essentially identical results. Regression of the values of  $K$  determined by the peak-slope method against those determined by the peak-rms method (for the same samples) yields  $y = 1.01x - 0.7$ , with  $r^2 = 0.92$ . The pooled standard deviation for replicate

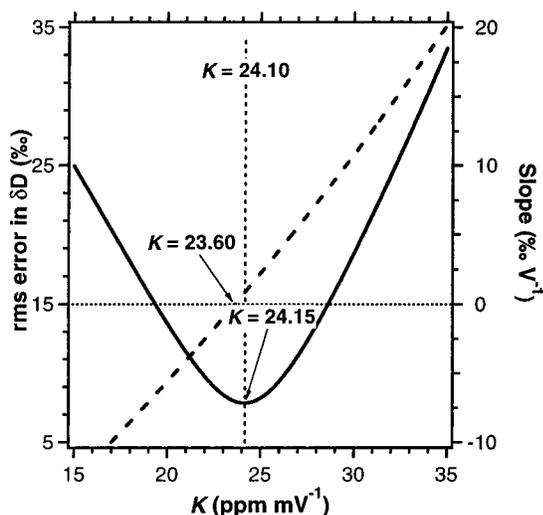


Figure 3. Comparison of  $H_3$  factors determined by three methods for a typical  $n$ -alkane analysis. Vertical dotted line gives the value of  $K$  obtained by the bellows method.  $K$  is determined by the peak-rms method as the minimum in the  $\delta D$  error vs  $K$  curve (solid line).  $K$  is determined by the peak-slope method where the slope of the regression of  $\delta D$  error on peak height is zero (dashed line).

measurements of  $K$  on a single day was  $0.64 \text{ ppm mV}^{-1}$  for the peak-rms method and  $0.77 \text{ ppm mV}^{-1}$  for the peak-slope method (53 degrees of freedom). These standard deviations do not differ significantly. Furthermore, both methods of calculating  $K$  can be used on the same data with little extra effort, and comparison of the results can provide insight into the performance of the analytical system.

Values of  $K$  determined by the peak-rms method are compared to those determined by the bellows method in Figure 4b. The standard deviation of replicate measurements of  $K$  using the bellows method was, in general, better than  $0.1 \text{ ppm mV}^{-1}$ . During the period January–March 1999, the two methods gave values for  $K$  that were usually indistinguishable. However, during January 2000, the bellows method produced values that were consistently 1–2 ppm lower than those obtained using the peak-rms method. In this case, systematic errors of up to 9‰ in the  $\delta D$  values of the  $n$ -alkanes were observed when the bellows method was used to determine the value of  $K$ . As expected, systematic errors were dependent on peak height mismatch between sample and standard peaks (Sessions et al., preceding paper), and were largest in pairs with the greatest mismatch. These systematic errors were absent when the peak-rms method was used to determine  $K$ . The peak-rms method, or other similar approaches, therefore, appear to be the more appropriate way to measure the value of  $K$  for isotope ratio monitoring measurements.

A more difficult question is *why* do the two methods produce different values? The only significant change in the analytical system between the two time periods over which we compared the two methods is that the pyrolysis furnace was rebuilt using a longer heated zone, slightly different geometry (during the former period the alumina tube rested beside the heating element and during the latter it was inside and concentric with the cylindrical heating element), and the addition of a small amount ( $\sim 5 \text{ mg}$ ) of Pt wire catalyst. Because the positioning of the thermocouple relative to the reactor tube changed slightly, it is possible that the temperature of the hot zone also changed slightly in the rebuilt

furnace. Separate experiments with much larger amounts of Pt (up to 500 mg) in the reactor have demonstrated a memory effect due to dissolution of  $H_2$  in the Pt metal, but the magnitudes of those effects were dependent on the D/H ratio of peaks rather than on peak size. A few measurements during the period January–March 1999 (when there was no Pt catalyst present) also produced significantly different values of  $K$  using the bellows and peak-rms methods (Figure 4b).

One possible explanation for the discrepancy between peak- and bellows-based values of  $K$  is that subtle changes in the pyrolysis reactor resulted in different levels of trace gases reaching the mass spectrometer, which in turn altered the magnitude of  $K$  during chromatographic runs. However, we have not been able to observe any measurable changes in the value of  $K$  over the course of a chromatographic run or over very large fluctuations in the partial pressures of  $CH_4$  and  $H_2O$  (see below). A second possible explanation is that some isotope-exchange process (or perhaps merely contamination) existed in the pyrolysis reactor, on metal fittings, in the GC, etc. If such an effect were dependent on peak size, it could account for the observed results. This potential explanation highlights the subtle question of what the “correct” value for  $K$  means. Any process that alters the D/H ratio of the  $n$ -alkane standard would likely also alter samples to a similar extent, so that even though the peak-rms method produced an incorrect value for  $K$ , its use would lead to correct  $\delta D$  values. Conversely, the bellows method might produce “correct” values for  $K$  but erroneous  $\delta D$  values.

**Dynamic Measurement of  $K$ .** The instantaneous method of measuring  $K$  is an attempt to combine the realistic conditions of the peak-based measurements with decreased time requirements. In theory, the use of changes in  $P_{H_2}$  across a single peak to measure the value of  $K$  is very attractive. The value of  $K$  could be determined separately for each peak in a chromatogram simply through appropriate data processing, without requiring any additional standards, chromatograms, or instrument time.

In practice, however, there are several difficulties with this approach. First, the time constants of the two signal pathways must be very closely matched for the instantaneous ion current ratio to reflect the D/H ratio of gas in the ion source. Even in the absence of  $H_3^+$ , mismatched time constants would result in large changes in the measured ion current ratios across a peak. The time constants can be closely matched by varying the feedback capacitance of the electrometers, but the time constants of both signal pathways are then determined by the slower mass-3 electrometer. When the time constants of the signal pathways are slower than the highest frequency contained in the peak being examined, the interaction between the nonlinearly changing ratio and the nonlinear damping of the time constants produces a pattern like that shown in Figure 5.

Numerical simulations of this phenomenon (using the approach described by Sessions et al., preceding paper) produce similar patterns, and suggest that at smaller amplifier time constants ( $RC < 0.3 \text{ s}$  for a Gaussian peak with  $\sigma = 2 \text{ s}$ ) this effect is minimized, with all the data falling on a straight line with slope =  $K$ . We were unable to obtain time constants faster than 1.3 s for our mass-3 electrometer due to its large feedback resistance ( $\sim 10^{12} \Omega$ ) in combination with unavoidable capacitance in the collector system, so this prediction remains untested. A

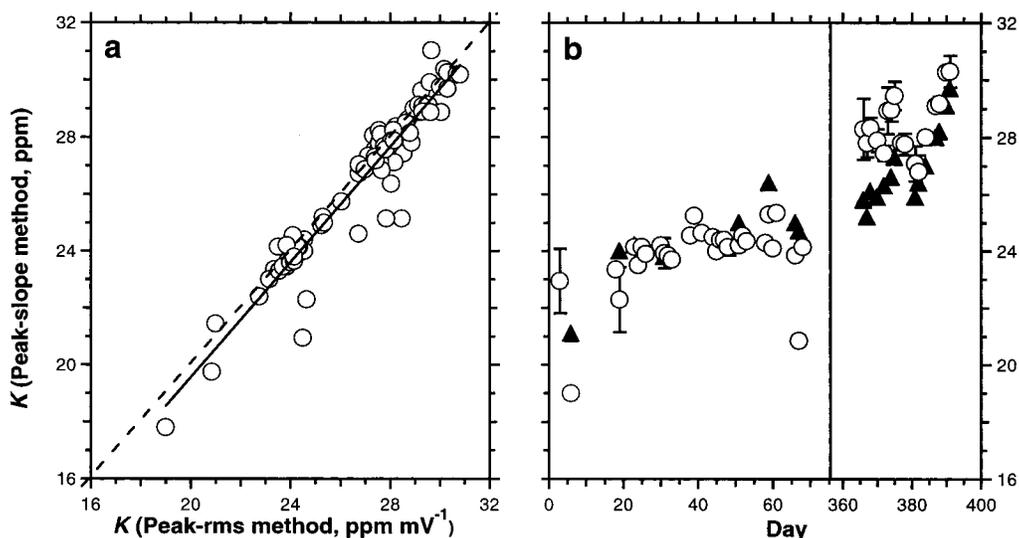


Figure 4. (a) Comparison of values of  $K$  determined for 105  $n$ -alkane analyses using the peak-rms and peak-slope methods. Solid line is the least-squares regression which has slope of 1.01. The light, broken line is  $y = x$ . (b) Comparison of values of  $K$  determined using the peak-rms (open circles) and bellows (triangles) methods over two separate periods of time. Error bars for the peak-rms method represent  $1\sigma$  for days on which three or more measurements were made. Error bars for the bellows method are the size of the symbols. Note the break in scale at 75 days.

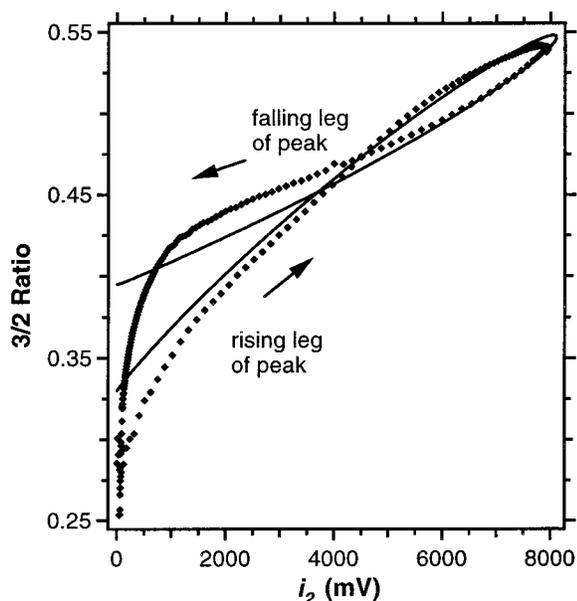


Figure 5. Instantaneous  $i_3/i_2$  ratios collected across a single peak. Each point represents an individual pair of background-corrected  $i_2$  and  $i_3$  measurements. Solid line is the calculated ratio for a Gaussian peak with width ( $4\sigma$ ) of 10 s, amplifier time constant of 1.3 s,  $K = 24$  ppm  $\text{mV}^{-1}$ , and true  $i_3/i_2$  ratio of 0.33.

possible alternative is to produce peaks that rise and fall very slowly, but the technical difficulty of producing those peaks negates the primary motivation for the instantaneous method, i.e., analytical convenience.

The second significant problem facing the instantaneous method is that the peak being examined must be isotopically homogeneous. Sample peaks from a GC will clearly be affected by isotope chromatography, with partial separation of the  $\text{H}_2$  and HD peaks. Even if deactivated capillaries are used to deliver reference peaks from an external peak generator, differences in the diffusivities of  $\text{H}_2$  and HD could lead to greater broadening of the  $\text{H}_2$  peak. Furthermore, differences in the pumping speeds

of  $\text{H}_2$  and HD in the ion source ( $^2\text{S}/^3\text{S} \approx (3/2)^{1/2}$ ) will tend to broaden the HD peak. If the latter two problems prove to be insignificant, the offset caused by isotope chromatography might be corrected by data processing, as is done prior to peak integration for irmGC/MS data.<sup>13</sup> Further examination of this approach seems well-justified, as the potential benefits in speed and accuracy are compelling.

**Uncertainties in the Measurement of  $K$ .** As described in the companion paper, relatively small errors in the value of  $K$  can result in significant errors in  $\delta\text{D}$  if the sample and standard peaks are not closely matched in both size and  $\delta\text{D}$  value. For example, a 1% error in the value of  $K$  would result in a  $\delta\text{D}$  error of  $> 1\%$  if the sample and standard are mismatched in height by only 2 V (see Figure 2 in Sessions et al., preceding paper). It is extremely important, therefore, to accurately assess uncertainties associated with measurements of the value of  $K$  and to propagate those uncertainties to resulting  $\delta\text{D}$  values.

Repeated measurements of  $K$  using the bellows method consistently give standard deviations which are  $< 0.1$  ppm, but as described above, the use of this method can lead to significant errors in measured  $\delta\text{D}$  values. On the other hand, if the source of the systematic errors can be identified and eliminated, it is likely to remain the most precise method of measuring  $K$ .

For the 21 days on which  $K$  was measured via the peak-rms method using at least three injections of the  $n$ -alkane standard, the daily standard deviation of those measurements ranged from 0.1 to 1.2 ppm and averaged 0.5 ppm. Given the typical magnitude for  $K$  of  $\sim 25$  ppm  $\text{mV}^{-1}$  during this period, 0.5 ppm  $\text{mV}^{-1}$  amounts to a 2% uncertainty in the value of  $K$ . Measuring the value of  $K$  with an uncertainty of  $< 1\%$  would therefore require 4 or more measurements of the  $n$ -alkane standard (i.e. standard error of the mean =  $\sigma/(n)^{1/2} = 0.25$ ). This is a very time-consuming requirement and provides great motivation for (1) determining the cause

(13) Ricci, M. P.; Merritt, D. A.; Freeman, K. H.; Hayes, J. M. *Org. Geochem.* **1994**, *21*, 561–571.

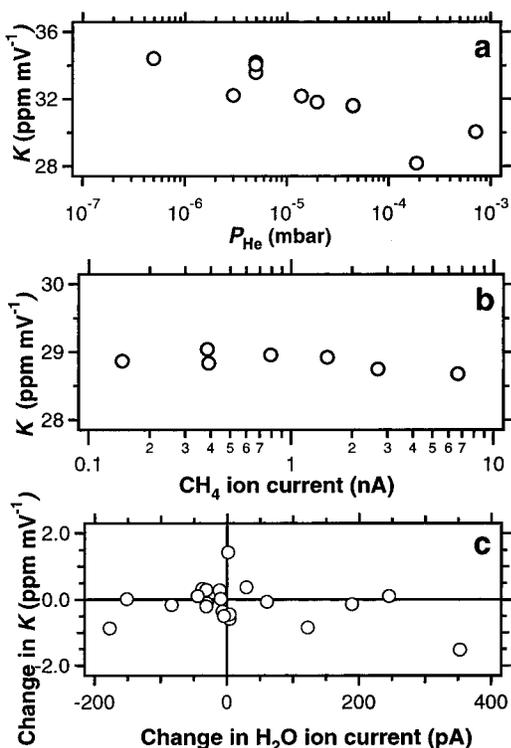


Figure 6. Changes in the value of  $K$  associated with He (a),  $\text{CH}_4$  (b), and  $\text{H}_2\text{O}$  (c) background signals. In panel c, relative changes in the value of  $K$  are plotted against changes in  $\text{H}_2\text{O}^+$  ion current over the same interval in order to remove the effects of instrumental drift.

of systematic errors in the bellows method or (2) improving electrometer time constants so that the instantaneous method might be used.

**Factors Controlling  $K$ .** We considered it possible that the systematic differences between values of  $K$  determined by the bellows versus peak-rms methods were due to subtle changes in the chemical composition of gases entering the IRMS ion source. To investigate this possibility, we examined changes in  $K$  with varying ion source pressure and varying partial pressures of  $\text{CH}_4$  and  $\text{H}_2\text{O}$ . Large increases in  $P_{\text{He}}$  result in small decreases in the value of  $K$ ,  $\sim 5\%$  for each 10-fold increase in  $P_{\text{He}}$  (Figure 6a). These changes are small enough that normal fluctuations in He flow rate, if buffered by an open split, should have virtually no influence on the value of  $K$ . The observed reduction in the rate at which  $\text{H}_3^+$  is formed may be due to a decrease in the number of  $\text{H}_2^+/\text{H}_2$  collisions due to dilution of the  $\text{H}_2$  by He.

No significant change in  $K$  was observed with changing pressures of  $\text{CH}_4$  (Figure 6b). This was unexpected, since  $\text{CH}_4$  can mediate proton transfer and is widely used as a reagent gas for chemical ionization mass spectrometry.<sup>12</sup> On the other hand, the presence of  $\text{CH}_4$  produces a substantial increase in mass-2 and mass-3 background signals. For example, a 0.25-nA  $\text{CH}_4^+$  ion beam is accompanied by 10 pA and 9 fA mass-2 and mass-3 ion beams, respectively. This “background” mass 3/2 ratio is  $\sim 30$ -fold higher than the D/H ratio of samples with a natural abundance of D. Thus, while the value of  $K$  might not change, the presence of large amounts of  $\text{CH}_4$  during an analysis (i.e., due to incomplete pyrolysis of sample components) could lead to substantial measurement errors (inaccurately high values of  $\delta\text{D}$ ).

Analysis of results obtained at varying partial pressures of  $\text{H}_2\text{O}$  is somewhat more complex, because up to 12 h was required for the  $\text{H}_2\text{O}$  pressure in the ion source to equilibrate with the carrier gas stream after the Nafion humidistat was adjusted. Changes in  $K$  due to  $\text{H}_2\text{O}$  partial pressure are therefore superimposed on changes due to instrument drift. To compensate for this, we compared relative changes in the value of  $K$  between consecutive measurements (generally 1–2 h apart) to changes in  $\text{H}_2\text{O}$  ion currents over the same period (Figure 6c). There is no correlation between changes in  $K$  and  $P_{\text{H}_2\text{O}}$ , indicating that water does not significantly affect the value of  $K$  at concentrations typical of isotope ratio monitoring analyses.

When background column bleed contributions cycled between 0 and 600 fA ( $m/z$  2 ion current) as column temperature varied between 40 and 325 °C, there were no measurable changes in the value of  $K$  (mean value,  $20.0 \pm 0.1$  ppm  $\text{mV}^{-1}$ ,  $n = 7$ ). Background mass spectra contain no ion currents that are clearly attributable to Si-containing compounds produced by the pyrolysis of GC column bleed.

In summary, the presence of  $\text{CH}_4$ ,  $\text{H}_2\text{O}$ , and GC column bleed in the analyte stream does not appear to enhance (or retard) the formation of  $\text{H}_3^+$ . Such effects could presumably occur through the transfer of protons to  $\text{H}_2$ , but this process is apparently insignificant at the partial pressures of  $\text{CH}_4$  and  $\text{H}_2\text{O}$  we examined. Other species such as CO and  $\text{N}_2$  may also be present in the ion source, but are even less likely to participate in proton-transfer reactions. It appears unlikely, therefore, that differences in the chemical composition of the analyte stream could account for the differences observed between the bellows and peak-rms methods of measuring  $K$ . On the other hand, these results offer some assurance that the value of  $K$  remains stable throughout GC analyses, despite small changes in carrier gas composition. This supports our observation (Sessions et al., preceding paper) that a single value of  $K$  can be used to accurately correct a wide range of peak sizes across a chromatogram even when very large temperature programs are employed.

## CONCLUSIONS

Approaches to accurate correction of  $\text{H}_3^+$  contributions during hydrogen isotope ratio monitoring mass spectrometry have been outlined (Sessions et al., preceding paper), but these calculations depend crucially on the accuracy and stability of the  $\text{H}_3$  factor ( $K$ ). A critical evaluation of several methods for determining the value of  $K$  indicates that static measurements of a range of  $\text{H}_2$  signals (the approach conventionally used in dual-inlet analyses) is highly precise ( $\sigma < 0.1$  ppm  $\text{mV}^{-1}$ ) but can lead to systematic errors in  $\delta\text{D}$  when applied to isotope ratio monitoring data. The source of these systematic errors is presently unknown. Peak-based measurements, on the other hand, produce values for  $K$  that are significantly less precise ( $\sigma \approx 0.6$  ppm  $\text{mV}^{-1}$ ) but which consistently produce more accurate  $\delta\text{D}$  values. Accordingly, the peak-based determinations of  $K$  are the most appropriate for use in isotope ratio monitoring. Determination of  $K$  from instantaneous D/H ratios measured across a single peak is theoretically possible and highly appealing, but would require that amplifier time constants be faster than can currently be achieved and that peaks be isotopically homogeneous. The uncertainty associated with measuring the value of  $K$  can limit the precision of the analytical

method unless sample and standard peaks are carefully matched in both size and  $\delta D$  value.

The rate of  $H_3^+$  production (i.e., the magnitude of  $K$ ) in our MAT-252 IRMS decreases by  $\sim 5\%$  for each order of magnitude increase in the partial pressure of He in the ion source. Given the very small variations in  $P_{He}$  typically encountered in systems in which the flow of He is regulated by an open split, the resultant changes in  $K$  would be undetectable. The presence of  $CH_4$  and  $H_2O$ , possible contaminants of the gas stream during nonideal operation of the pyrolysis reactor, has no significant effect on  $K$ . Likewise, the introduction of GC column bleed has no measurable effect on  $K$ . We conclude that isotope ratio monitoring analyses do not require additional measures beyond those used in conventional dual-inlet analyses to stabilize the  $H_3$  factor.

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