Determination of stable carbon and hydrogen isotopes of light hydrocarbons

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Determination of Stable Carbon and Hydrogen Isotopes of Light Hydrocarbons

Ingolf Dumke,* Eckhard Faber, and Jürgen Poggenburg

Federal Institute for Geosciences and Natural Resources (BGR), Hannover, FRG

A combined system for the measurement of $^{13}C/^{12}C$ and D/H ratios on light hydrocarbons (C_1–C_3) and CO_2 is described. The system is designed for natural gas and sediment gas analyses. It comprises gas chromatographic separation with online combustion of hydrocarbons to CO_2 and H_2O, reduction of H_2O to H_2 on zinc in closed ampules, and mass spectrometric determination of isotope ratios ($\delta^{13}C$, δD) using a mass spectrometer inlet system especially designed for low hydrogen gas quantities. Isotope analyses can be carried out in the range of 3–10000 μL of CO_2 and 100–10000 μL of H_2 (gas quantities converted from sample compounds during preparation, STP). Including all preparation steps, reproducibility of isotope values for large sample quantities (>100 μL of produced CO_2 and >1000 μL of produced H_2) is ±0.2‰ for $\delta^{13}C$ and ±3‰ for δD. With decreasing gas quantities the reproducibility is lower, e.g. ca. ±1‰ for $\delta^{13}C$ (at 5–10 μL) and ca. ±7‰ for δD (at 100–150 μL). The isotope values tally with data of other laboratories (±0.2‰ for $\delta^{13}C$).

INTRODUCTION

Stable isotope ratios of light hydrocarbons are often successfully used in oil and gas exploration. The $^{13}C/^{12}C$ ratio of methane is the most widely used parameter for the genetic classification of naturally occurring hydrocarbon gases in terms of isotope values. It allows the distinction between bacterial and thermal hydrocarbons (1–4) and is related to the generation mechanisms and to the maturity of source rocks of thermal gases, respectively (5–7). Schoell (2) and Whiticar et al. (8) have shown that additionally the $^2H/^1H$ ratio of methane in combination with the $^{13}C/^{12}C$ ratio characterizes different pathways of bacterial methane formation. Furthermore carbon isotope values of higher homologues (ethane,

propane) can be related to source rock maturities as well (9). They also show up mixtures of thermal gases and allow the isotope data of the mixing partners and related source rock maturity data to be calculated (10).

Isotope data have been measured on quite different types of samples including reservoir gases, headspace and desorbed gases from drill cuttings, cores from deep wells, and near surface sediments. Reviews on possible applications and published literature can be found elsewhere (7, 9, 11, 12).

The determination of isotope ratios requires an analytical system including gas chromatography, quantitative combustion, water reduction, and mass spectrometry. In contrast to the wide-ranging literature on applications of light hydrocarbon isotope data, only a small amount of literature is available on details about the analytical technique. For carbon isotope analysis a circulation combustion system with cupric oxide and oxygen (13) has been widely used for methane and other organic compounds for various investigations (1, 14–17). Silverman and Oyama (18) describe gas chromatographic separation coupled with an online combustion for carbon isotopic determination on methane as an automated sampling and preparation system for use in microbial metabolism studies.

Up to four components (C_1, C_2, C_3, and CO_2), separated on a gas chromatographic column, can be prepared for ^{13}C analysis with a system published by Faber (19) and Faber and Stahl (20). At the BGR first measurements on D/H ratios on natural gas samples were carried out in 1976 (21). Deuterium analysis on methane especially designed for low-volume samples has been developed by Faber et al. (22). The system described below is the continued development of these earlier techniques and allows the determination of carbon and hydrogen isotopic ratios on methane, ethane, and propane and the carbon isotopic ratio of CO_2 from one gas sample within one preparation cycle. The capability of isotope analysis in
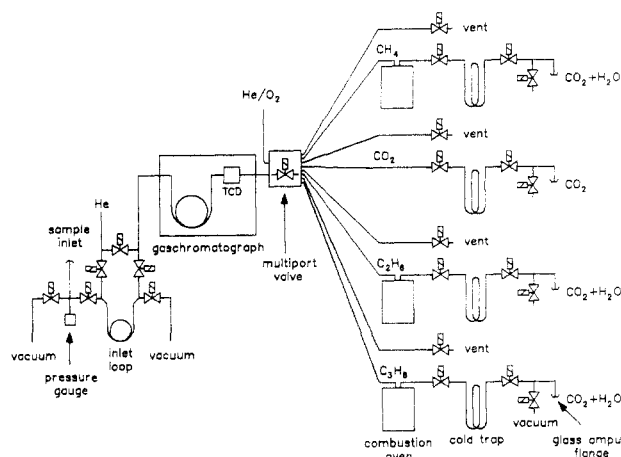
Figure 1. Scheme of the gas preparation line (for detailed explanation see text).
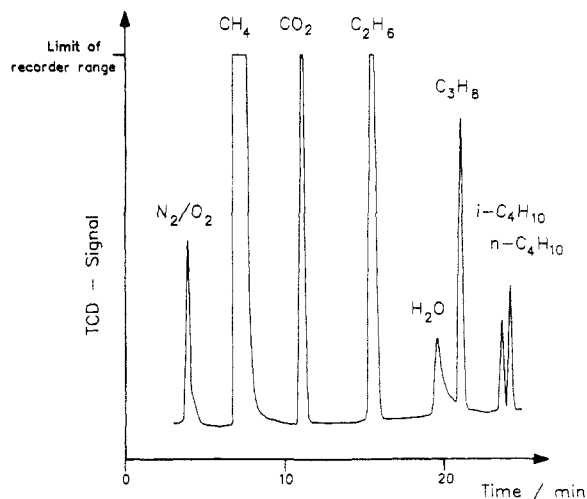

Figure 2. Chromatogram of the laboratory standard gas E245 (for composition see Table II, no valve switching).

a wide range of hydrocarbon concentrations, including very low gas quantities, makes it suitable for natural gases as well as headspace and shallow sediment gases.

EXPERIMENTAL SECTION

For the determination of stable isotope ratios, hydrocarbons have to be converted to CO_2 and H_2 required by stable isotope mass spectrometers. The complete technique can be divided into three parts, which are put into effect independent from each other: a gas preparation line to separate the desired components by gas chromatography with online combustion of hydrocarbons to CO_2 and H_2O, a reduction system to convert the combustion water to H_2, and stable isotope mass spectrometers.

Gas Preparation. A scheme of the gas preparation line is shown in Figure 1. The whole range of sample pressures from several atmospheres (test gases) down to some 10 mbar (sediment gases) can be handled by the evacuable inlet system with easily changeable inlet loops of different sizes. Loop volumes of 3, 10, and 30 mL ($^1/_4$ in. o.d. stainless steel) have been found to be useful. An additional 3-mL loop packed with Porapak Q allows transfer of all the hydrocarbons from a sample vessel into the inlet loop by freezing the latter in liquid nitrogen, a significant procedure for small samples with hydrocarbon quantities near the lower detection limit.

Helium is the carrier gas for separation on the column ($^1/_4$ in. o.d. stainless steel, 2.5 m length, Porapak Q) and passes the hydrocarbon components through the combustion lines. A thermal conductivity detector (TCD) monitors the gas components N_2/O_2, CH_4, CO_2, C_2H_6, H_2O, C_3H_8, i-C_4H_{10}, n-C_4H_{10}, i-C_5H_{12}, n-C_5H_{12}. Figure 2 shows a typical gas chromatogram (laboratory standard gas E245, see below). N_2 and O_2 as well as higher hydrocarbon
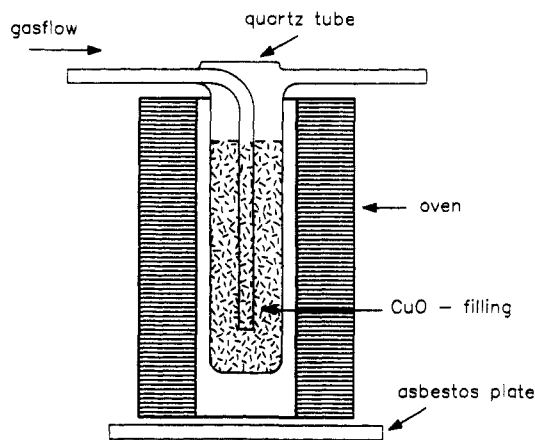

Figure 3. Sectional drawing of the combustion oven.

homologous (C_{5+}) are not fully resolved. Unsaturated compounds (C_2H_4 and C_3H_6) may occur shortly before but well separated from the corresponding saturated compounds. The chromatogram is not used for quantitative evaluation because correct peak integration is impeded by extra peaks and base-line shifts due to necessary valve switching during the preparation cycle.

The column is temperature programmed from -50 °C (2 min initial time) to 220 °C (10 min final time) with rise of 15 °C/min for 8 min followed by 5 °C/min for another 8 min and again 15 °C/min until the final temperature is reached. This temperature program assures separation of the early compounds air (N_2/O_2), methane, and CO_2.

Experiences with earlier systems (20) have shown that it is essential to eliminate memory effects occurring when the various hydrocarbon components are combusted successively in the same oxidation line. Therefore the desired components (methane, ethane, and propane) are separated into individual pathways at the exit of the chromatographic column. A multiport valve (VALCO type AH2 CSC-8-HPA) switches the carrier gas to one of three separate combustion lines and an additional cold trap for CO_2. Vent valves prevent passing of blank contributions or undesired components (e.g. unsaturates) through a combustion line. Complete removal of combustion products from the oven chamber is achieved by a second He flow which is connected to all outlet ports of the multiport valve except the one momentarily linked to the gas chromatograph. This flow path can be also used with oxygen for daily regeneration of the oxidation catalyst.

The construction of the electrical combustion furnace (manufactured by H.-D. Buchwald, Bolsehle 55, 3071 Husum, FRG) is shown in Figure 3. The vertical arrangement saves space and avoids gaps in the flow path which may occur due to shrinking of the CuO filling. Copper(II) oxide (CuO) is the most popular catalyst for oxidation of hydrocarbons and has been extensively studied, mainly because of its application in elemental analyzers. Ebel (23) has given a good review on this subject. Oxidation of methane has been examined by Kainz and Horvatitsch (24), Horacek et al. (25), Pechanec (26), and also by Matthews and Hayes (27). Methane, mostly the major component within samples considered here, is known to be difficult to combust quantitatively and requires relatively high combustion temperatures. The necessary temperature depends on the type of grain size of the CuO used. Wire form CuO has been chosen here because it allows the use of a considerable amount in the oven to combust several large samples (some 10 mL of methane) without regeneration and does not lead to unacceptable high flow resistance. Temperature is chosen at 880 °C.

Cold traps to trap combustion products (CO_2 and H_2O) at liquid nitrogen temperature are made of glass (Duran, 6 mm o.d.). No undesired effects (loss of CO_2, isotopic shifts) caused by the procedure of freezing both combustion products in one cold trap have been observed. In fact, bulk of CO_2 and H_2O freeze well displaced onto the tube walls due to the temperature gradient in the first part of the cold trap. This may support a quantitative separation. Combustion products are individually transferred to glass ampules (6 mm o.d., 15 cm length) through heated transfer lines using cryogenic separation (dry ice/2-propanol mixture, ca.
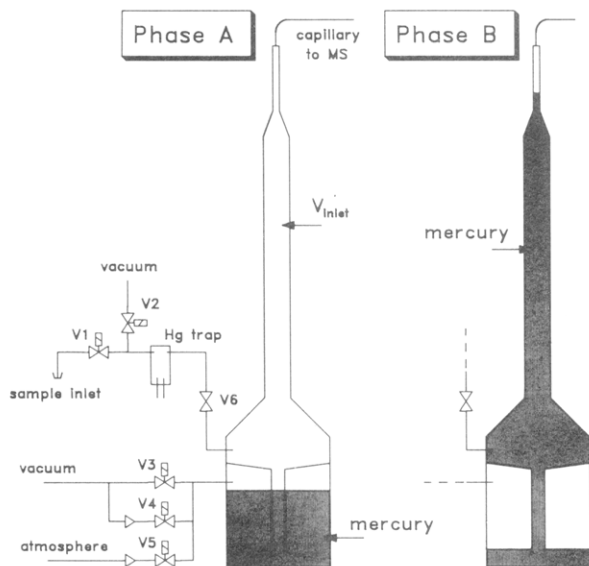
Figure 4. Scheme of the mass spectrometer inlet system: V_1–V_6, valves (for functions see text); V_{inlet}, inlet volume; phase A, maximum inlet volume for sample inlet; phase B, inlet volume compressed by rising mercury for pressure adjustment after sample inlet.

−80 °C). The glass ampules are sealed by torch.

A rotary pump and a turbo molecular pump are attached to the system. Except for the cold traps all tubes are made of stainless steel ($^1/_{16}$ in. or $^1/_4$ in.). High vacuum tight, air-actuated bellows valves (NUPRO SS-4BK-1C) have been chosen for all evacuable parts of the system. All valves in the system can be either manually switched or activated by computer. The whole process, starting with inlet of the sample and ending with the collection of all combustion products in the cold traps, runs automatically controlled.

Water Reduction. Methods of reducing water to hydrogen for isotope analysis are mostly based on the reaction with hot uranium in flow systems (28, 29) or zinc in flow systems (30) or closed ampules (31, 32).

Because sample amounts as small as possible should be processed, flow systems were regarded as disadvantageous due to their large internal volumes and surface areas and the risk of memory effects. Therefore a further development of the method described by Coleman et al. (32) has been chosen. The combustion water is transferred to glass reaction tubes with a filling of some granules of cleaned zinc. After the reaction tube was heated at 450 °C for at least 2 h it contains zinc oxide and molecular hydrogen and can be used directly at the mass spectrometer for isotope ratio determination. The cleaning procedure described by Coleman et al. is applied to prepare the zinc granules which are then kept under vacuum until usage. Besides the zinc charge indicated by Coleman et al. (BDH AnalaR shot 0.5–2.0 mm) another charge (Riedel-de-Haen, Zink feingranuliert pa. 31651) has also been found to be usable for the reduction process.

Mass Spectrometry. The determination of isotope ratios is carried out on commercial isotope mass spectrometers. $^{13}C/^{12}C$ ratios are determined on a Finnigan MAT 250 with which the whole range of sample quantity, ca. 3 to ca. 10.000 μL of CO_2 (STP), can be handled with standard equipment using cool finger technique below the quantity of 50 μL CO_2. $^2H/^1H$ determinations are performed on a Finnigan MAT Delta D. With standard equipment the dynamics of volume variation in the inlet system is not suitable for H_2 gas quantities down to less than 100 μL (STP). Therefore a special inlet system for the sample side has been constructed which uses a mercury piston for volume variation (Figure 4). The volume in the upper part (V_{inlet}, ca. 250 mL) is separated from the mercury reservoir in the lower part. V_{inlet} is evacuable via valve V2 and the float valve V6 (phase A) provided that the headspace above the mercury is evacuated via valves V3 or V4 too. After sample inlet via valve V1 the mercury can be raised by carefully floating the lower chamber via valve V3 (phase B). The rising mercury automatically closes the float valve V6. The maximum volume of V_{inlet} is large compared to the volume

Table I. Calibration Data Set for Deuterium Analysis (in Parts per Mil)

standard	δ_{WST}[a]	δ_{SMOW}[b]	$\delta_{SMOW\text{-}SLAP}$[c]	nominal value
V-SMOW	95.3 ± 0.1	0.0	0.0	0.0
NBS 1	46.0 ± 0.3	−45.0	−46.0	−47.1
NBS 1a	−101.3 ± 0.3	−179.5	−183.4	−183.2
GISP	−107.3 ● 0.5	−185.0	−189.1	−189.8
SLAP	−363.4 ● 1.8	−418.8	−428.0	−428.0
	$\delta_{SMOW/WST}$[d] = −87.0		f_{corr} = 1.022	

[a] δ_{WST}, δ value versus working standard. [b] δ_{SMOW}, δ value versus V-SMOW. [c] $\delta_{SMOW\text{-}SLAP}$, δ value recalculated to the SMOW-SLAP scale using f_{corr}. [d] $\delta_{SMOW/WST}$, δ value of the working standard versus V-SMOW.

Table II. Composition of Laboratory Standard Gas E 245

component	concn, vol %
nitrogen	1.2
carbon dioxide	3.1
methane	89.5
ethane	3.9
propane	1.2
isobutane	0.3
n-butane	0.4
isopentane	0.2
n-pentane	0.1

of sample tube and inlet flange so that more than 95% of the sample is available in V_{inlet} for compression. The maximum volume can be decreased by a factor of ca. 500. This allows pressure adjustment and measurement of samples down to ca. 30 μL of H_2 (STP). The system is made of stainless steel except for the glass standpipe to watch the mercury piston. The standpipe is surrounded by a plexi tube for security. The float valve V6 consists of a free up and down movable Teflon cylinder with silicon cap which is pressed against a ring edge by the raising mercury. The total height of the system is 85 cm so that undesired flushing of the lower chamber will not result in pushing the mercury into the capillary of the mass spectrometer. Valves V4 and V5 are operated electrically with interceptions to allow careful adjustment of the mercury level. Via valve V3 quick pumping down of the mercury is possible. Malfunction of V6 resulting in mercury spill toward V1 and V2 is indicated by electrical contacts within a mercury trap right before V6. The whole inlet system has been manufactured by EMG (Elektronik-Mechanik-Gerätebau GmbH, 2800 Bremen 61, FRG).

RESULTS

Isotope ratios are reported in the commonly used δ notation

$$\delta \text{ value} = \frac{R_{sample} - R_{standard}}{R_{standard}} *1000 \ (\permil) \qquad (1)$$

where R is the ratio $^{13}C/^{12}C$ or $^2H/^1H$, respectively. Results are given according to the PDB scale for $\delta^{13}C$ (33) and according to the SMOW-SLAP scale for δ^2H (δD) (34).

Water Reduction. The apparatus described has been tested with common international water standards (Table I). A calibration of the H_2 gas working standard at the mass spectrometer according to V-SMOW has been established. As a common method of eliminating systematic deviations from the nominal values, a correction factor according to the SMOW-SLAP scale (34) has been determined. Delta values recalculated to the SMOW-SLAP scale correspond to the nominal values (34, 35). While the reproducibility of delta values of consecutive samples was mostly better than ±0.5‰, a long-term reproducibility in routine analysis of ±1–2‰ seems to be realistic.

Gas Preparation. Reproducibility. Long-term reproducibility of the system can be deduced from repeated routine
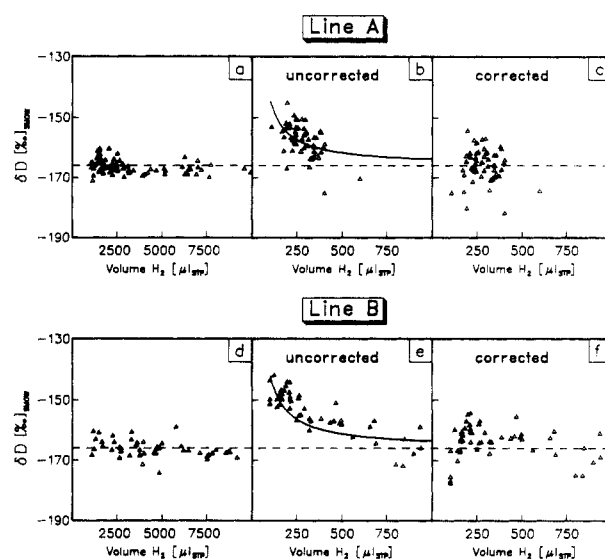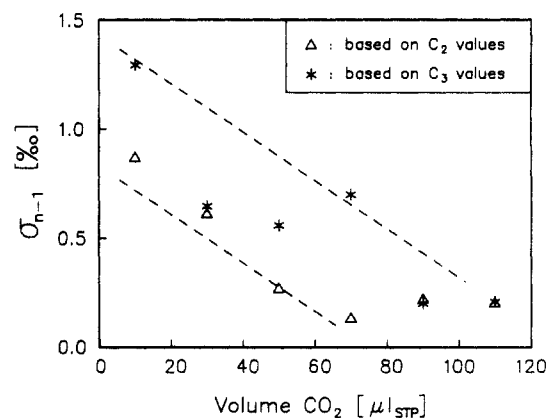
Table III. Isotope Ratios (Mean Values of Multiple Analyses, in ‰) and Standard Deviations (σ_{n-1}) for the Gas Standard E 245

	methane	ethane	propane	carbon dioxide	sample vol, μL_{STP}
$\delta^{13}C_{PDB}$	-44.5 ± 0.13	-32.0 ± 0.25	-29.0 ± 0.25	no values	>100
	a	a	a	-10.4 ± 0.7	<100
$\delta D_{SMOW\text{-}SLAP}$	-166 ± 2.4	b	b		>1000
	-166 ± 5.5	-126 ± 6.6	-118 ± 6.6		<1000

^{a} See text and Figure 7. ^{b} No values.
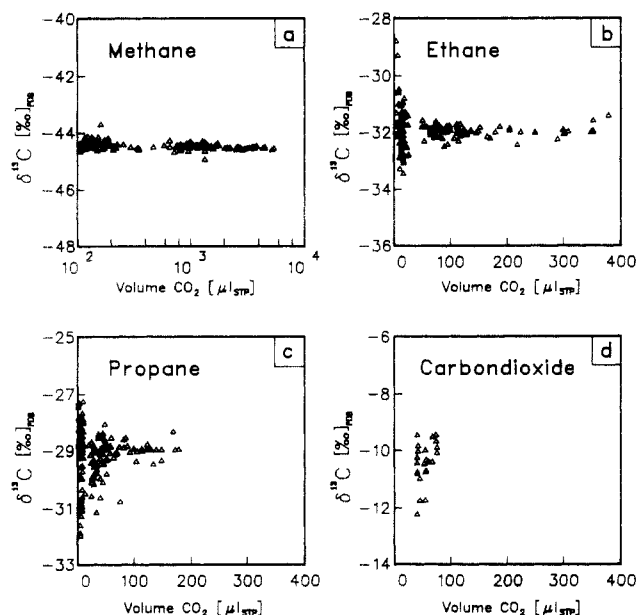
Figure 5. Measurements of $\delta^{13}C$ for methane, ethane, propane, and carbon dioxide of the laboratory standard gas E245 as a function of sample volume (μL of CO_2 produced in preparation, STP).

Figure 6. Measurements of δD for methane (laboratory standard gas E245) as a function of sample volume (μL of H_2 produced in preparation, STP) for two different preparation lines (A and B): dotted lines, nominal value, mean of data displayed in graphs a and b; solid lines (graphs b, e), correction curves used for blank correction of measured δ values for sample quantities below 1000 μL of H_2.

Figure 7. Reproducibility of $\delta^{13}C$ values (expressed as standard deviation σ_{n-1}) as a function of sample volume (μL of CO_2 produced in preparation, STP).

measurements on a laboratory standard. The gas (Laboratory-No. E 245, composition given in Table II) has been used for daily check of the system and Figures 5–7 display results of preparations spread over a time interval of about 2 years. Figure 5 illustrates the measurement of $\delta^{13}C$ for methane, ethane, propane, and CO_2 as a function of sample volume (CO_2 produced in preparation, STP), ranging from some microliters up to about 6 mL. It can be seen that down to ca. 100 μL of produced CO_2 the scattering of the isotope values stays fairly constant while below 100 μL it increases with decreasing sample volume.

Regarding corresponding measurements for δD of methane in Figure 6, a different situation can be found. Two data sets of two different GC–combustion lines (A and B) used at BGR are presented. Sample volumes (H_2 produced in preparation, STP) range from ca. 50 μL up to ca. 10 mL. Down to ca. 1000 μL (Figure 6a,d) scattering is quite homogeneous and a mean value of $\delta D = -166‰$ can be calculated. Below 1000 μL (Figure 6b,e) scattering increases and in addition the measured isotope values rise with decreasing sample volume. This is explained by a blank of water in the GC–combustion system which contributes to each sample during the preparation. Sources for this blank are traces of water and hydrocarbons in the He carrier gas and traces of compounds that remain on the GC column and in the combustion oven from previous preparations and are given off slowly. The H_2 blank contribution during a preparation cycle has been determined to be ca. 30 ± 10 μL with a δD value of about $-95 \pm 14‰$ for line A. Mean values for line B are 20 μL with δD of $-47‰$. This illustrates that the blank contribution strongly depends on the condition of the combustion lines. However, experience shows that the blank contribution stays constant within given limits for quite long time periods. By use of formula 2 for

blank correction on δD values for H_2 quantities below 1000 μL the distribution of Figure 6c,f shows about the same mean values as for the large volume samples

$$\delta D_{corr} \sim \frac{\delta D_{MS} V_{mS} - \delta D_{blank} V_{blank}}{V_{MS} - V_{blank}} \qquad (2)$$

with δD_{corr} the corrected delta value, δD_{MS} the measured delta value at the mass spectrometer, V_{MS} the volume of H_2 determined at the mass spectrometer (real sample volume plus blank contribution), δ_{blank} the delta value of blank contribution ($=-95‰$), and V_{blank} the volume of blank contribution ($=30$ μL). For CO_2 the blank contribution of a preparation cycle

Table IV. Isotope Values for Natural Gas Standards (NGS1/2/3)[a]

institute code	NGS1			NGS2				NGS3
	C_1	C_2	C_3	C_1	C_2	C_3	CO_2	C_1
Results of an Interlaboratory Comparison ($\delta^{13}C_{PDB}$ in ‰)								
F-6	-28.9	-26.4	-19.9	-43.5	-31.1	-24.3	-7.5	-73.1
I-4	-29.16	-26.02		-44.92	-32.1	-25.9	-8.08	-72.85
NL-1	-29.16	-25.42		-44.76	-31.17		-8.02	-72.67
USA-15	-28.91	-25.93		-44.57	-32.06	-25.7		-72.77
USA-16	-28.97	-26.09	-21.67	-44.58	-31.84	-25.01	-8.38	-72.55
USA-17	-28.6	-26.3		-43.1	-31.7	-23.5	-8.9	-69.6
Evaluation of Data								
mean	-28.95	-26.03		-44.24	-31.66	-24.88	-8.19	-72.26
σ_{n-1}	±0.21	±0.35		±0.75	±0.43	±1.0	±0.51	±1.3
After Outlier Correction								
mean	-28.95	-26.09		-44.71	-31.93	-25.00	-8.16	-72.79
σ_{n-1}	±0.21	±0.16		±0.17	±0.19	±0.7	±0.19	±0.21
BGR Results								
$\delta^{13}C_{PDB}$	-28.9	-25.9	-22.9	-44.6	-31.9	-25.5	-8.1	-72.9
$\delta D_{SMOW-SLAP}$	-140	-114		-178	-125			-178

[a] Results of an interlaboratory comparison, published by HUT (36), and measurements at the BGR.

has been determined to be in the order of 0.1–0.2 μL (STP) which is neglectable compared to sample volumes.

A summary of the results with the laboratory standard E245 is given in Table III and Figure 7:

$\delta^{13}C$ isotope ratios can be reproduced by ±0.2‰ for CO_2 quantities of >100 μL (corresponding to 100 μL of methane, 50 μL of ethane, and 33 μL of propane, respectively), whereas below 100 μL the reproducibility decreases according to Figure 7 to ca. ±1‰ at 5–10 μL.

δD isotope ratios can be reproduced by ±3‰ for H_2 quantities of >1000 μL (corresponding to 500 μL of methane, 333 μL of ethane, and 250 μL of propane, respectively), whereas below 1000 μL the reproducibility decreases to ca. ±7‰ at 100–150 μL.

Gas Preparation. Accuracy. The accuracy of the system can be checked by measuring samples with known isotope values. Unfortunately, gas standards with internationally accepted isotope values have not existed up to now. But in 1985 three natural gas samples of different composition and origin were supplied by the International Atomic Energy Agency (IAEA), Vienna. Results of $\delta^{13}C$ isotope analyses of several laboratories were published by HUT (36). Table IV shows these results. Mean and outlier corrected mean (excluding values beyond $2\sigma_{n-1}$) have been calculated and can be compared to results obtained by the BGR preparation system described here. The latter coincide with the outlier corrected means for all measured components within the limits of standard deviation. A major deviation does exist for propane of sample NGS1, but only two considerable differing values measured by other laboratories are available. Isotope ratios for deuterium were not published by HUT (36) but the BGR data are included in Table IV.

CONCLUSION

With the method described it is possible to process hydrocarbon components for $\delta^{13}C$ and δD isotope analysis in the range of 3–10000 μL of produced CO_2 and 100–10000 μL of produced H_2, respectively. The reproducibility and accuracy are sufficient to genetically classify hydrocarbons from reservoirs (large gas quantities) and sediment samples (small gas quantities). The lower limit of processable gas quantities is not given by mass spectrometry but by the blank contribution in the gas preparation and the water reduction system. Blank correction, necessary for low-volume hydrogen isotope measurements cannot be applied to lower quantities than ca. 100 μL of H_2 because of the steep slope of the correction curve

in this region. Further improvement of hydrogen isotope measurements therefore has to concentrate mainly on reducing the system blank. The fact that correction is possible by using the measured system blank allows the conclusion that the water reduction process does work down to these very small sample amounts without severe systematic error.

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Evidence for Radical Anion Formation during Liquid Secondary Ion Mass Spectrometry Analysis of Oligonucleotides and Synthetic Oligomeric Analogues: A Deconvolution Algorithm for Molecular Ion Region Clusters

J. A. Laramée, B. Arbogast, and M. L. Deinzer*

Department of Agricultural Chemistry and the Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon 97331

It is shown that one-electron reduction is a common process that occurs in negative ion liquid secondary ion mass spectrometry (LSIMS) of oligonucleotides and synthetic oligonucleosides and that this process is in competition with proton loss. Deconvolution of the molecular anion cluster reveals contributions from $(M - 2H)^{\bullet-}$, $(M - H)^-$, $M^{\bullet-}$, and $(M + H)^-$. A model based on these ionic species gives excellent agreement with the experimental data. A correlation between the concentration of species arising via one-electron reduction [$M^{\bullet-}$ and $(M + H)^-$] and the electron affinity of the matrix has been demonstrated. The relative intensity of $M^{\bullet-}$ is mass-dependent; this is rationalized on the basis of base-stacking. Base sequence ion formation is theorized to arise from $M^{\bullet-}$ radical anion among other possible pathways.

INTRODUCTION

Reduction of analytes during analysis by liquid secondary ion mass spectrometry (LSIMS) became a topic of discussion almost as soon as the analytical technique itself was introduced. Not all compounds yielded exclusively preformed $(M - H)^-$ or $(M + H)^+$ cluster ions as first predicted by the original models to describe the ionization phenomenon (*1, 2*). It was shown that chlorophylls with extended π systems gave 3.4 times more $M^{\bullet-}$ than $(M - H)^-$ (*3*) and *N*-carboxymethyl-3-dicyanomethylene-5-nitroindolin-2-one yielded exclusively $M^{\bullet-}$ (*4*) under particle bombardment. Positive LSIMS of nucleosides (*5*) and some peptides (*6*) yields enhanced $(M + n)^+$ ions that are not explained by the isotopic composition. It was suggested that in these cases low-lying unoccupied molecular orbitals (LUMOs) were available for capture of low-energy electrons to produce $M^{\bullet-}$ ions (*7*) or reduced multiprotonated $(M + n)^+$ ions (*5*).

Extensive reduction has been reported for nucleotides (*8*), cationic triarylmethane and Oster dyes (*9*), diquaternary ammonium and other organic salts (*4, 10*), anthracyclines and anthracyclinones (*11*), quinones and tetracyanoquinodimethanes (*4*), or generally for compounds expected to undergo facile reduction in solution. Charge-transfer complexes also

have been proposed as sources of $M^{\bullet-}$ and $M^{\bullet+}$ pairs (*12, 13*).

In addition to being a plausible source of thermalized electrons (*7*), the matrix provides radical species and possibly hydrogen atoms through high-energy particle bombardment (*14, 15*). Alternatively, it has been suggested that hydrogen atoms may be abstracted from matrix molecules by radical ions (*16*) as is more typical of free radicals in solution, and some reduction of organic salts and complexes may even occur in glycerol without particle bombardment (*10*). Thus the nature of the matrix significantly affects the degree of reduction of sample molecules. Organic dyes in tetraglyme and diethanolamine (*9*), and Met-enkephalin in thioglycerol (*6*), for example, show $(M + 1)^+$ cluster ion patterns that are closer to that expected from the isotopic composition than when these compounds are analyzed in glycerol.

A serious deficiency in dealing with reduction reactions in LSIMS is the lack of a quantitative treatment for the ions being observed. This is important if isotope patterns are used to confirm empirical formulas, or if fragment-ion-forming processes are needed to understand the structural features of the molecule. It is particularly critical when molecular weights of larger molecules are relevant. We have developed a computational method for deconvoluting the ion clusters that allows a quantitative determination of the sample ion composition that was analyzed. This has brought about some new findings about the relative importance of the electron affinities of matrix and sample molecules being analyzed.

EXPERIMENTAL SECTION

Chemicals. The oligodeoxynucleotide carbamate analogues were synthesized as described previously (*17*). Amino functionality on adenine and cytosine was blocked with the benzoyl (Bz) group and on guanine was blocked with the phenylacetyl (PA) group. Backbone linkages consisting of carbamate and *N*-methylcarbamate were examined while terminal 5′ amino groups were blocked with monomethoxytrityl (mmT) and trityl (Ph₃C) groups, respectively. Structural groups corresponding to dA, dG, dT, and dC represent the carbamate analogues to mononucleotides. Phosphodiester oligonucleotides d(pCGCG), d(pT)₆, d(pT)₈, and d(pT)₁₀ were obtained from Sigma Chemical and were used without further purification; d(ACGT), d(AGCT), d(T)₆, d-(CCGTGG), d(T)₇, and d(T)₈ were synthesized in-house by fol-