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The potential for application of ink stable isotope analysis in questioned document examination

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ABSTRACT

We investigated a novel application of stable isotope abundance analysis of nitrogen (^{15}N), carbon (^{13}C), hydrogen (^{2}H), and oxygen (^{18}O) to characterize pen ink. We focused on both ballpoint and gel pen inks. We found that the isotope ratios of ink from pens purchased together in a package were similar and within-package stable isotope ratio variability was not significantly larger than the variability of isotope reference materials used during analysis. In contrast, the isotope ratios of ink from pens of the same brand purchased in three states of the continental USA were significantly different from each other and there was isotope ratio variation among pens of the same brand but different, unknown production periods. The stable isotope ratios of inked paper were statistically distinguishable using measured $\delta^{15}N$ values. Paper inked with different gel pens was statistically distinguishable using measured $\delta^{2}H$ values. The capacity of stable isotope ratios to differentiate among ballpoint inks as well as gel inks shows that stable isotope analysis may be a useful and quantifiable investigative technique for questioned document examination, although current sample size requirements limit its utility. Application of the technique in casework will require the development of micro-scale sampling and analysis methods.

1. Introduction

Understanding the authenticity and origins of documents is of widespread commercial and legal interest. As such, questioned document examination (QDE) is an important forensic discipline because it focuses on answering questions related to a document's history [1,2]. Examiners often investigate paper documents or records to determine authenticity, such as the Hitler diaries "discovered" in East Germany in 1983 [3] or counterfeit currency surfacing in the global marketplace [4]. Document examination can also be useful in investigations of material origin, as when the FBI searched for the origins of the 2001 Amerithrax letters [5].

Questioned document examination is a comparative science in which trained examiners observe characteristic features of two or more samples in order to compare and contrast different documents. Casework investigations may involve scrutiny of the applied media (ink) and the substrate (paper), both of which can be examined using a variety of non-destructive and destructive analysis techniques. Non-destructive examination often relies on optical observation via microscopy, photography, and additional spectral analysis methods [6–9]. Destructive QDE techniques typically rely on chemical analysis methods; examples include chromatography [10,11], elemental analysis [12], and mass spectrometry [13–15].

Chromatography—specifically, high performance thin-layer chromatography (TLC)—is among the most common destructive analysis techniques used in QDE for identifying ballpoint pen inks because analysis is relatively straightforward and results are rapidly generated [16–19]. Yet the identification of gel pen inks via TLC is often not possible [20]. This is because gel pen inks contain pigment-based colorants as opposed to the dye-based colorants found in most ballpoint pen inks [21]; these pigments are not soluble in the organic solvents associated with TLC analysis methods. Given the reduced potential for tampering, gel ink pens are marketed as an appealing choice for signing documents like wills or contracts. As such, document examiners frequently encounter gel inks during investigations [20]. However, authentication of text written with a gel ink pen is challenging because of (1) the difficulty in removing pigmented ink from the substrate once it is applied and (2) the lack of validated chemical analysis techniques to distinguish different gel inks. Several methods for characterizing and differentiating gel inks have been investigated [20,22–24], but to date no single approach has been deemed preferable for the discrimination and identification of gel ink pens.

In this study we explored a novel chemical analysis technique for pen inks: stable isotope ratio analysis. One of the key features of stable isotope analysis is the ability to relate and distinguish chemically identical materials through measurement of naturally occurring small

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differences in the stable isotope abundances within the materials. Stable isotope analysis can provide an additional isotope "fingerprint" to further characterize a piece of evidence. Measurement of stable isotope ratios has previously proven useful in the investigation of many different materials of forensic interest [25–27]. For example, stable isotope analysis has been used to differentiate packaging tapes [28,29] and paints [30] as well as drugs [31–33] and explosives [34–36]. Related to QDE, recent work by Jones et al. focused on the stable isotope analysis of commercially available office paper and demonstrated that carbon isotope ratios could be used to discriminate different papers [37–39].

Here we show the potential for stable isotope analysis to be applied as a quantitative testing method for characterizing pen inks during questioned document examination. We first investigated ink isotope ratio variation within packages of pens, by purchase location, and among pens of various ages before measuring the stable isotope ratios of pen inks surveyed in one location. We then focused on the stable isotope analysis of inks on paper. Finally, we present some limitations of the technique as explored in this work and discuss future work to further develop stable isotope analysis for application in QDE casework.

2. Materials and methods

2.1. Stable isotope analysis and statistical analysis

Samples were analyzed for nitrogen (N), carbon (C), hydrogen (H), and oxygen (O) element concentrations and stable isotope ratios. Stable isotope ratios are reported in δ-notation, where $\delta = R_A/R_{STD} - 1$ and R_A and R_{STD} are the ratios of the rare to common stable isotopes (e.g., $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{2}H/^{1}H$, and $^{18}O/^{16}O$) in the sample and an international standard, respectively. The international standard for nitrogen is atmospheric air (AIR); for carbon, it is Vienna-Pee Dee Belemnite (V-PDB). The international standard for both hydrogen and oxygen is Vienna-Standard Mean Ocean Water (V-SMOW). Stable isotope ratios reported as δ-values are presented in "per mil" on the basis of (for example) $-4.56 \times 10^{-3} = -4.56‰$.

Stable isotope ratios in samples were measured using a MAT 253 isotope ratio mass spectrometer (ThermoFinnigan; Bremen, Germany). For the measurement of nitrogen and carbon, samples were weighed as described below and then introduced to an elemental analyzer (EA; ThermoFinnigan) via a zero-blank autosampler (Costech Analytical; Valencia, California, USA). For the measurement of hydrogen and oxygen, samples were weighed as described below and then introduced to a high temperature conversion elemental analyzer (TC/EA; ThermoFinnigan) via a zero-blank autosampler.

Samples were analyzed alongside two primary laboratory reference materials that were used to (1) correct for potential memory, time, and/or peak area effects during the analytical sequence and (2) normalize measured sample data to international isotope scales. Another laboratory reference material was analyzed as an unknown; comparison of this secondary reference material's corrected δ-value to its calibrated δ-value was used to verify corrections and normalization. All samples within an analytical sequence were reanalyzed if the secondary reference material did not correct/normalize to within analytical uncertainty of its calibrated δ-value. Laboratory reference materials used for carbon and nitrogen isotope analysis were glutamic acids. Hydrocarbon reference materials were used for hydrogen isotope analysis and benzoic acids were used for oxygen isotope analysis.

Primary glutamic acid laboratory reference materials were calibrated using the international isotope reference materials USGS40 and USGS41 to give reference values for $\delta^{15}N_{AIR} = +49.63‰$ and –4.56‰ and $\delta^{13}C_{VPDB} = +23.96‰$ and $-28.18‰$ for UU-CN-1 and UU-CN-2, respectively. The primary laboratory hydrocarbon reference materials were calibrated using the international isotope reference materials IAEA-CH-7, V-SMOW, and SLAP to give reference values for $\delta^{2}H_{VSMOW} = -35.8‰$, and $-248.7‰$ for n-C_{24} and n-C_{28}, respectively. Benzoic acid primary laboratory reference materials were calibrated against the international isotope reference materials IAEA-601 and IAEA-602 to give reference values of $\delta^{18}O_{VSMOW} = +37.02‰$ and $-3.71‰$ for UU-OH-5 and UU-OH-7, respectively. Analytical precision—defined as the standard deviation of the secondary laboratory reference material—was 0.22‰ for N (UU-CN-3; $\delta^{15}N_{AIR} = +9.30‰$, $n = 40$), 0.06‰ for C (UU-CN-3; $\delta^{13}C_{VPDB} = -12.35‰$, $n = 40$), 3.5‰ for H (PARA; $\delta^{2}H_{VSMOW} = -99.8‰$, $n = 33$), and 0.41‰ for O (UU-OH-6; $\delta^{18}O_{VSMOW} = +26.15‰$, $n = 34$).

Statistical analyses were completed using Prism for Mac OS X (Version 5.0c, GraphPad Software Inc.; La Jolla, California, USA). The significance level for all statistical tests was set at $\alpha = 0.05$. When samples were analyzed more than once, replicate analyses were averaged and the mean value used in tests.

2.2. Collection of inks

To investigate the isotope ratio variability among pens, we purchased 1 package each of black, blue, and red ballpoint pens of two common brands (Bic® and Papermate®) in Utah, USA. In addition, we purchased 1 package of black ballpoint pens of the same two brands in New Hampshire and Louisiana, USA. The black ballpoint pens from three different states were also used to investigate isotope ratio variation based on purchase location. To investigate the isotope ratio variation among inks of different ages, we collected 7 Bic® Round Stic® ballpoint pens from the assortment of pens accumulated at one author's home.

We also purchased a variety of ballpoint and gel ink pens in June–August 2012 from a large office supply store and a university bookstore located in Salt Lake City, Utah, USA. Pens were purchased both individually and as packages. We purchased various combinations of ink types, brands, and colors to collect a total of 27 ballpoint ink pens and 19 gel ink pens.

To extract all the ink from pens, we removed the ink reservoir from the pen barrel and then removed the pen tip from the reservoir. The reservoir was placed inside a 12-ml round-bottom Exetainer® vial (Labco Limited; Lampeter, Ceredigion, United Kingdom). When present, we trimmed the "grease plug" [21] from the gel pen reservoir. The reservoir was centrifuged at 720 RCF for 5 min to pool ink at the bottom of the test tube. We assumed centrifugation homogenized the ink sample. The reservoir was discarded and the ink was stored in the capped vial (placed upright in the lidded boxes used for shipping the Exetainer® vials) at room temperature.

2.3. Preparation of inks for stable isotope analysis

Samples were weighed by dipping a metal wire into the ink, then touching the wire to the bottom of a 3.5 × 5.0 mm capsule (Costech Analytical). We initially weighed ~2 mg of each ink into tin capsules for nitrogen and carbon analysis and ~0.3 mg of each ink into silver capsules for hydrogen and oxygen analysis. Initial mass was recorded to the nearest 0.001 mg. Capsules were left open and placed in a brass rack that was heated at 40 °C for a minimum of 24 h to allow the ink to dry. After drying, capsules were weighed again then crimped closed and analyzed, as described above, to determine element concentrations.

From the initial and final mass data, we calculated mass loss associated with drying. Using the mass loss and element concentration data, we reweighed all inks so that gas peak areas generated by the combustion or pyrolysis of dried inks would be consistent within an analysis sequence. Initial masses ranged from 0.75 to 2.17 mg for carbon and nitrogen analysis and from 0.050 to 0.300 mg for hydrogen and oxygen analysis. Reweighed inks were analyzed, as described above, to determine stable isotope ratios. All ballpoint and gel pen inks were analyzed once each for carbon, nitrogen, hydrogen, and oxygen element concentrations and stable isotope ratios; 5 of the 19 purchased gel pens were analyzed in duplicate for hydrogen and oxygen element concentrations and stable isotope ratios.

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2.4. Preparation of ink on paper for stable isotope analysis

Before ink extraction, 5 black ballpoint ink pens and 3 black gel ink pens were used to "color" one side of small rectangular pieces of standard office copy paper ~7 mg in mass (about 11 × 9 mm) for nitrogen isotope analysis and ~0.3 mg in mass (about 3 × 2 mm) for hydrogen and oxygen isotope analysis. Inked paper pieces were folded and then placed inside tin or silver capsules that were crimped closed. For comparison, we also sampled and analyzed blank pieces of paper of the same masses from the same page (OfficeMax® 30% recycled copy paper; purchased in Salt Lake City, UT, USA).

The hydrogen and oxygen element concentration and stable isotope ratios of the paper pieces were measured as described above for ink. For nitrogen analysis of paper pieces, the perchlorate trap for collecting water between the EA and the mass spectrometer was replaced with a trap containing Ascarite® II in addition to magnesium perchlorate. The Ascarite® II (Sigma Aldrich; St. Louis, Missouri, USA) adsorbed CO_2 from the combustion products generated by the EA leaving only N_2 for stable isotope ratio analysis. This allowed us to combust large masses of paper, thereby maximizing the N_2 peak area while avoiding CO_2 saturation of the detectors. No timing changes were required to the instrument method because the replacement trap was the same volume as the original trap.

3. Results

3.1. Within-package isotope ratio variation of pen ink

We used the stable isotope ratio data from 10 packages of ballpoint pens to calculate the within-package standard deviation (SD) for each of the four measured elements (N, C, H, and O). Standard deviations were 0.05‰–0.15‰ for δ^{15}N, 0.02‰–0.15‰ for δ^{13}C, 0.9‰–3.3‰ δ^{2}H, and 0.26‰–0.98‰ for δ^{18}O values (Table 1). We compared these within-package standard deviations to the standard deviations of the laboratory reference materials used to correct and normalize measured isotope ratio data (shown in Table 1). The within-package standard deviation of δ^{15}N values was significantly lower than the standard deviation for the reference materials ($P < 0.001$). For δ^{13}C, δ^{2}H, and δ^{18}O values, there was no statistically significant difference between the within-package variances and the reference material variances.

From these data, we concluded that inks from pens within the same package had similar isotope ratios. As a consequence, we analyzed only a single pen from each of the other packages purchased in the survey. Stable isotope ratios for the pens used to quantify within-package variation were averaged and treated as a single observation in the remainder of the study.

Table 1
Isotopic variation measured for ink collected from ballpoint pens that were purchased together in a package. Also shown is the isotopic variation for laboratory reference materials (RMs) used during stable isotope ratio analysis. Within-package ink variations were statistically compared to reference material variations using an F-test. Variations that were not significantly different are noted with "NS".

Purchase state	Pen brand	Ink color	n	Within-package standard deviation (‰)			
				δ^{15}N	δ^{13}C	δ^{2}H	δ^{18}O
New Hampshire	Bic®	Black	9	±0.15	±0.15	±3.3	±0.56
Louisiana	Bic®	Black	9	±0.08	±0.09	±0.9	±0.73
Utah	Bic®	Black	10	±0.09	±0.03	±2.2	±0.98
Utah	Bic®	Blue	9	±0.07	±0.09	±1.5	±0.47
Utah	Bic®	Red	10	±0.10	±0.08	±2.8	±0.26
New Hampshire	Papermate®	Black	8	±0.07	±0.07	±1.9	±0.40
Louisiana	Papermate®	Black	10	±0.08	±0.03	±1.9	±0.35
Utah	Papermate®	Black	12	±0.05	±0.05	±1.4	±0.48
Utah	Papermate®	Blue	9	±0.15	±0.08	±1.3	±0.64
Utah	Papermate®	Red	10	±0.08	±0.02	±1.0	±0.44
Average for pens				±0.09	±0.07	±1.8	±0.53
Average for RMs				±0.20	±0.06	±1.5	±0.33
F-test results				$P < 0.001$	NS	NS	NS

3.2. Location-dependent isotope ratio variation in pen ink

We used the stable isotope ratio data from 6 packages of black ballpoint pens—purchased in New Hampshire, Louisiana, and Utah—to investigate location-dependent differences in two brands of pens (Bic® and Papermate®). Package means are presented in Table 2. Within brand, locations were compared using 1-way ANOVA with Tukey's HSD post-hoc test. The mean δ^{15}N, δ^{13}C, δ^{2}H, and δ^{18}O values were significantly different between the three purchase locations for both Bic® and Papermate® black ballpoint pens ($P < 0.0001$ in all cases); although individual F-values for the six ANOVA tests are not shown, post-hoc test results for all pairwise comparisons are indicated in Table 2. Bic® pens purchased in New Hampshire and Louisiana were indistinguishable based on measured δ^{13}C and δ^{18}O values. All other pairwise comparisons of Bic® pens purchased in three states were significant and all pairwise comparisons of Papermate® pens purchased in three states were significant.

3.3. Isotope ratio variation in pen ink of unknown ages

To investigate the isotope ratio variation in ink of unknown ages, we selected 7 black ballpoint pens of the same brand and type (Bic® Round Stic®) from the collection of pens available at the Salt Lake City, UT residence of one of the authors. Because these pens had been acquired at various (unknown) times, we used the pens to investigate the general effect of age/time on ink isotope ratios. We measured the stable isotope ratios of ink collected from these pens; data are presented in Table 3. The stable isotope ratios varied, with ranges of 15.6‰ for δ^{15}N values, 1.4‰ for δ^{13}C values, 32‰ for δ^{2}H values, and 2.2‰ for δ^{18}O values (Table 3). In direct comparison, the ranges for δ^{15}N and δ^{13}C values in 7 pens collected from one author's house were larger than those observed for packages of Bic® pens purchased from three states within a 3-month period (Table 2).

3.4. Elemental concentration and isotope ratio variation in different pen inks

The individual N, C, H, and O concentrations measured in this study were summed to calculate an aggregate element concentration for each ink sample. Aggregate element concentrations ranged from 85% to 100% for ballpoint inks ($n = 41$) and from 80% to 97% for gel inks ($n = 18$; data not shown). The median aggregate element concentration of ballpoint inks was significantly higher than gel inks (Mann–Whitney test; $P < 0.0001$, $U = 123.0$). Considering each element concentration individually, ballpoint inks had a significantly higher median %C value (71% vs. 47%; $P < 0.0001$, $U = 13.00$) and gel inks had a significantly higher median %O value (14% vs. 30%; $P < 0.0001$, $U = 17.50$).

Table 2
Within-package mean isotope ratios of black ink collected from two brands of ballpoint pens, purchased in three states. Means for each isotope were compared using 1-way ANOVA with a Tukey's HSD post-hoc test ($\alpha = 0.05$); δ-values within a brand that share a superscript letter are not significantly different.

Purchase state	Pen brand	Ink color	n	Package mean isotope ratio (‰)			
				δ^{15}N	δ^{13}C	δ^{2}H	δ^{18}O
New Hampshire	Bic®	Black	9	-16.0^{a}	-29.6^{a}	-55^{a}	$+16.8^{a}$
Louisiana	Bic®	Black	9	-16.9^{b}	-29.5^{a}	-59^{b}	$+16.1^{a}$
Utah	Bic®	Black	10	-14.7^{c}	-29.0^{b}	-105^{c}	$+18.3^{b}$
		Range		2.2	0.6	50	2.2
New Hampshire	Papermate®	Black	8	0.0^{a}	-31.1^{a}	-66^{a}	$+14.5^{a}$
Louisiana	Papermate®	Black	10	-0.6^{b}	-31.6^{b}	-84^{b}	$+16.5^{b}$
Utah	Papermate®	Black	12	-3.2^{c}	-31.5^{c}	-91^{c}	$+17.7^{c}$
		Range		3.1	0.5	25	3.2

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Table 3
Measured isotope ratios of black ink collected from 7 ballpoint pens of the same brand (Bic®) and type (round barrel, with cap), but different, unknown ages. Pens were collected from one author's residence.

Sample identity	Measured isotope ratio (‰)			
	δ^{15}N	δ^{13}C	δ^{2}H	δ^{18}O
Exhibit #1	−14.1	−30.1	−41	+14.1
Exhibit #2	−5.2	−31.0	−68	+14.5
Exhibit #3	−11.7	−30.8	−56	+16.2
Exhibit #4	+0.2	−30.9	−68	+16.1
Exhibit #5	−2.4	−30.8	−73	+15.8
Exhibit #6	+1.5	−31.5	−62	+16.3
Exhibit #7	ND	ND	−66	+16.1
Maximum	+1.5	−30.1	−41	+16.3
Minimum	−14.1	−31.5	−73	+14.1
Range	15.6	1.4	32	2.2

ND = not determined.

Figs. 1 and 2 show the variations of isotope ratios for N, C, H and O measured for all samples of black, blue, and red ballpoint and gel inks, respectively, analyzed in this study. Stable isotope ratios for surveyed ballpoint pens had a total range of 20.6‰ for δ^{15}N values, 5.0‰ for δ^{13}C values, 101‰ for δ^{2}H values, and 12.8‰ for δ^{18}O values (Fig. 1). Measured stable isotope ratios for purchased gel pens had a total range of 9.8‰ for δ^{15}N values, 5.3‰ for δ^{13}C values, 97‰ for δ^{2}H values, and 31.4‰ for δ^{18}O values (Fig. 2). Standard deviations of gel pens analyzed in duplicate ($n = 5$) were 0.1‰–1.9‰ for δ^{2}H values and 0.04–0.73‰ for δ^{18}O values (data not shown). There were significant differences in median δ-values between the two ink types for C ($P < 0.0001$, $U = 86.00$), H ($P < 0.01$, $U = 195.0$), and O ($P < 0.01$, $U = 213.5$). There was no significant difference in the median δ^{15}N values of ballpoint and gel inks, based on a Mann–Whitney test.

3.5. Isotope ratio analysis of ink on paper

To investigate the feasibility of stable isotope analysis of ink on paper without the removal of ink from the page, we "colored" pieces of copy paper with black ballpoint and gel ink pens. By solidly inking the pieces of paper, rather than writing on them, we maximized the amount of applied ink available for analysis. This approach approximated the use of pens to complete optical answer sheets (e.g., Scantron® forms).

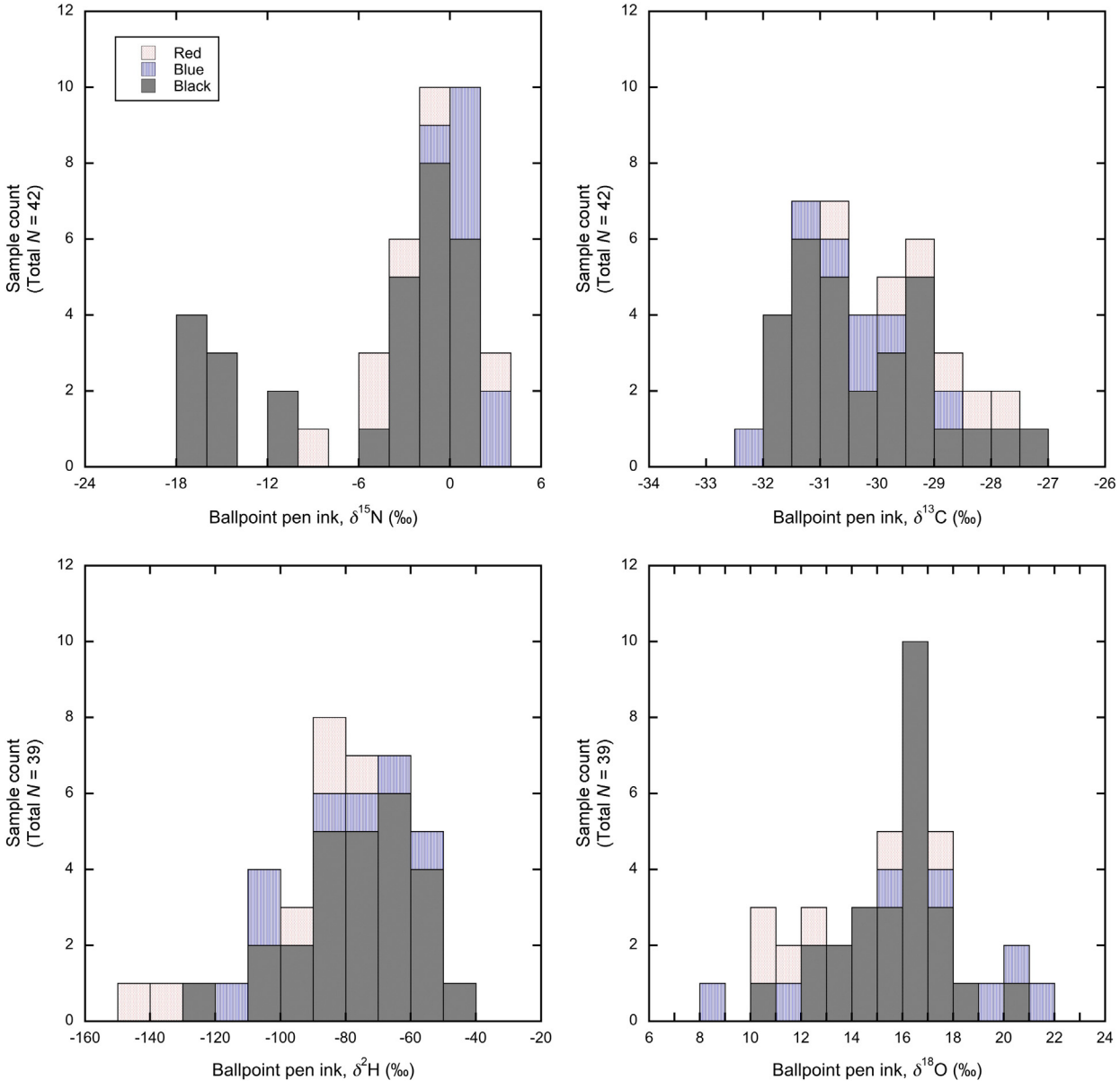

Fig. 1. Four histograms of the measured nitrogen, carbon, hydrogen, and oxygen stable isotope ratios of red, blue, and black inks collected from ballpoint pens in this study.

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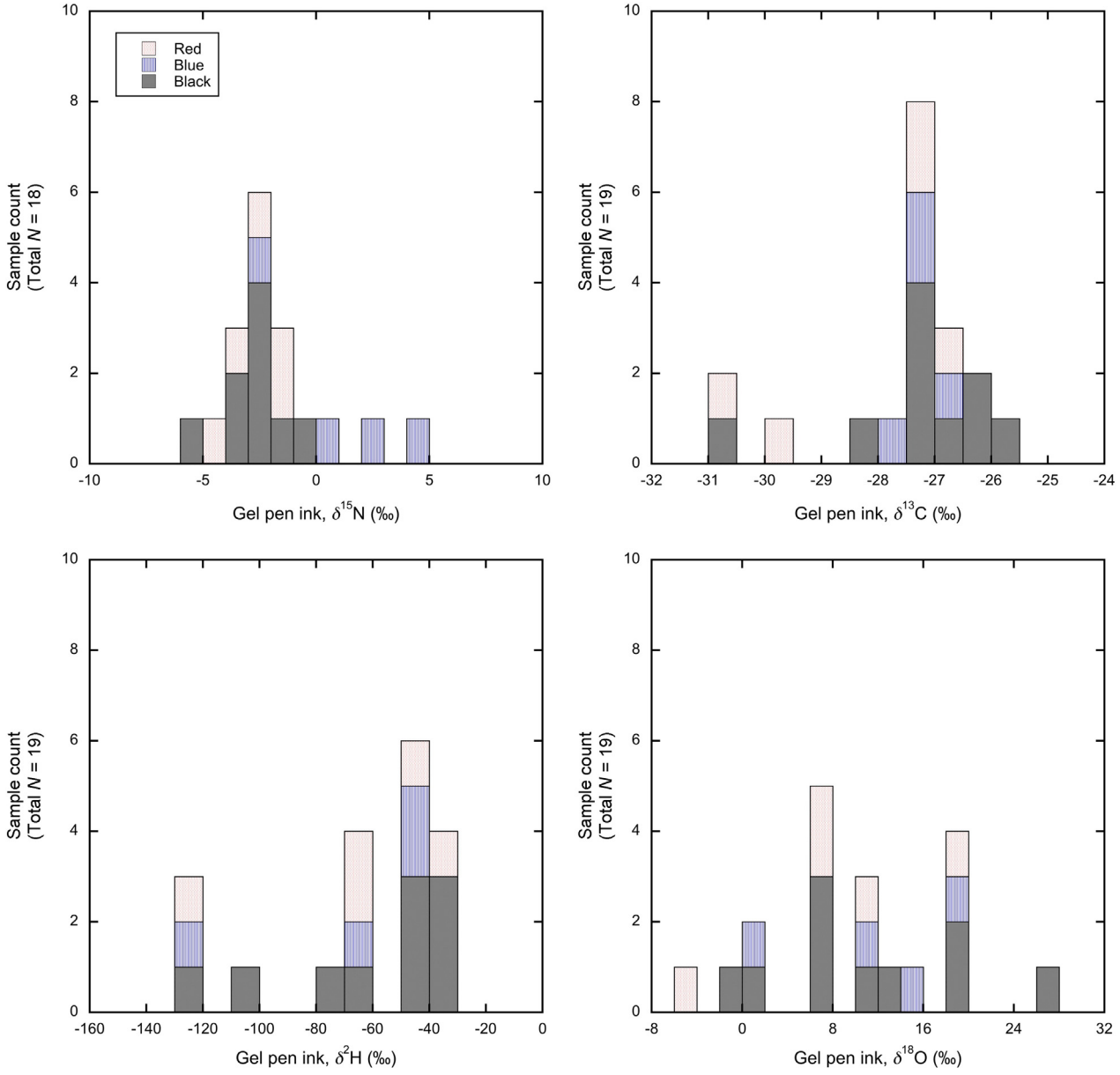

Fig. 2. Four histograms of the measured nitrogen, carbon, hydrogen, and oxygen stable isotope ratios of red, blue, and black inks collected from gel pens in this study.

Ballpoint inks on paper were analyzed for nitrogen isotope ratios because the observed natural variation in ballpoint ink $\delta^{15}N$ values was relatively large (Fig. 1). Due to the large range $\delta^{18}O$ values observed for surveyed gel ink pens (Fig. 2), gel inks on paper were measured for $\delta^{18}O$ (and $\delta^{2}H$) values in addition to $\delta^{15}N$ values. Data are shown in Table 4. Also presented in Table 4 are the stable isotope ratios of the inks measured separately from the paper and of the blank pieces of paper measured without ink.

The $\delta^{15}N$ values of ink measured alone were significantly different than the $\delta^{15}N$ values measured for the ink on paper (paired t-test, $t = 2.754$, $P = 0.283$); this was true for both ballpoint and gel pens (Table 4). This difference was likely due to the relatively small—but significant—contribution of N in the paper to the combined ink and paper sample.

The $\delta^{15}N$ values of the paper pieces colored with ballpoint ink and the blank paper were compared using a 1-way ANOVA with Tukey's HSD post-hoc test ($F = 866.8$, $P < 0.0001$); post-hoc test results are indicated in Table 4. All 5 ballpoint pen inks on paper were distinguishable

Table 4
Measured isotope ratios of black ink from 5 ballpoint pens and 3 gel pens analyzed alone and once applied to paper. Also shown are the measured isotope ratios of the blank paper. For each isotope, inks on paper were compared to one another and to blank paper using 1-way ANOVA with a Tukey's HSD post-hoc test ($\alpha = 0.05$); δ-values within a pen type that share a superscript letter are not significantly different.

Sample	Pen type	$\delta^{15}N$ (‰)		$\delta^{2}H$ (‰)		$\delta^{18}O$ (‰)	
		Alone	On paper	Alone	On paper	Alone	On paper
Pen 1	Ballpoint	−14.7	-10.4^{a}	−105	ND	+18.3	ND
Pen 2	Ballpoint	−11.9	-8.6^{b}	−120	ND	+12.3	ND
Pen 3	Ballpoint	−2.4	-1.5^{c}	−73	ND	+11.0	ND
Pen 4	Ballpoint	−0.5	0.0^{d}	−67	ND	+20.5	ND
Pen 5	Ballpoint	−15.0	-12.1^{e}	−101	ND	+17.4	ND
Paper	N/A	N/A	$+0.2^{d}$	N/A	ND	N/A	ND
Pen 6	Gel	−2.2	-1.5^{a}	−44	-90^{a}	−1.5	$+21.4^{a}$
Pen 7	Gel	−2.1	$-2.1^{a,b}$	−41	-87^{b}	+1.6	$+21.7^{a,b,c}$
Pen 8	Gel	−2.7	-2.5^{b}	−37	-84^{c}	+18.4	$+22.3^{b}$
Paper	N/A	N/A	-0.6^{c}	N/A	-89^{a}	N/A	$+22.4^{b,c}$

ND = not determined, N/A = not applicable.

from one another based on nitrogen isotope ratios; with one exception, all ballpoint pen inks were also distinguishable from blank paper. For that exception (Pen 4), the $\delta^{15}N$ value of the ballpoint ink measured alone (−0.5‰, Table 4) was similar to that of blank paper used in the test (+0.2‰).

We used a 1-way ANOVA with Tukey's HSD post-hoc test to compare the δ-values of the paper pieces colored with gel ink and the blank paper; individual *F*-values are not shown ($P < 0.05$ in all cases), but post-hoc test results are indicated in Table 4. All paper pieces colored with gel ink had $\delta^{15}N$ values significantly lower than the blank paper used in the test (i.e., lower than −0.6‰, Table 4). Pens 6 and 8 on paper were distinguishable based on $\delta^{15}N$ values. Pens 7 and 8 on paper were distinguishable from blank paper based on measured hydrogen isotope ratios. All 3 gel pen inks on paper were statistically distinguishable from one another based on $\delta^{2}H$ values (Table 4). Despite the large range in $\delta^{18}O$ values for the 3 gel pens measured alone, only Pen 6 on paper was significantly different from Pen 8 on paper and from blank paper.

4. Discussion

4.1. Characterization of pen ink using stable isotope ratio analysis

In this study, we explored the novel application of stable isotope analysis in questioned document examination for the characterization of ballpoint and gel inks. We showed it was possible to measure the stable isotope ratios of N, C, H, and O "as is" in all surveyed samples, establishing that this analysis technique could be broadly applied to both ballpoint and gel inks. We are unaware of stable isotopes ratio studies on pen inks—or, alternatively, other dyes or pigments—and we were thus unable to compare our data with any published datasets. However, we note that the ranges of isotope ratios we measured for black, blue, and red ballpoint and gel inks (Figs. 1 and 2) are within the realm of isotope ratios measured for other organic materials [40,41]. This is not unexpected given that most ink ingredients are organic compounds, including colorants derived from methane, naphthalene, and aldehydes, and colorant vehicles mixed from alcohols, glycols, and water [42].

The lower aggregate element concentration of N, C, H, and O for gel inks as opposed to ballpoint inks suggests that gel inks contain additional elements that could be useful for sample characterization and discrimination. This is supported by literature showing that gel inks contained several trace elements in sufficient quantities for analysis (i.e., Al, Cu, Sr, and Zn) [24,43]. Beyond measurement of trace element concentrations within an ink sample, it may be possible to analyze the stable isotope ratios of some of rare elements—such as Sr—found in the ink.

In addition to stable isotope ratio analysis "as is," we also showed that it was possible to analyze ink after its application to paper (Table 4). Analysis of ink on paper demonstrated that paper could significantly contribute to the total measured δ-value of a sample. This was not surprising for $\delta^{2}H$ and $\delta^{18}O$ values as paper is a hydrocarbon. However, we did not expect to observe significant amounts of nitrogen in paper. Although the paper contributed a small fraction of the nitrogen in the total measured $\delta^{15}N$ value of an inked paper piece, the majority of the nitrogen signal in a combined ink and paper sample came from ink itself. Most ink vehicles contain glycol and water [42,44] while ink additives are typically hydrocarbons [45]. We therefore assume the nitrogen source in the ink was primarily colorant (dyes and/or pigments) and not the ink vehicle or additives.

To assess the usefulness of ink stable isotope ratio analysis in QDE, we must understand both the specificity and fidelity of a measured isotope ratio "fingerprint" for an ink sample and the extent of isotope ratio variation among different brands and types of ink. We showed that ink from pens purchased in the same package had similar isotope ratios and packages were characterized by small isotope ratio variability (Table 1), suggesting constancy in production of the ink found in pens that were sold together. In contrast, however, we discovered that pens of the same brand purchased in three locations had significantly different isotope ratios (Table 2). Interestingly, the $\delta^{2}H$ values of black ink from ballpoint pens purchased in Utah were lower than those measured for the same types of pens purchased in New Hampshire and Louisiana. This pattern was more pronounced for the Bic® pens than the Papermate®; the difference in $\delta^{2}H$ values (as well as $\delta^{15}N$ and $\delta^{18}O$ values) between the purchase locations suggests that the Bic® pens purchased from New Hampshire and Louisiana may represent one manufacturing batch or origin while the pens purchased in Utah represent another batch or origin. If the inks were manufactured near to the location of pen purchase, then the lower $\delta^{2}H$ values for the Utah inks could be due to the isotope ratio composition of water used during ink manufacture, as water from inland and high elevations regions (like Utah) is characterized by lower $\delta^{2}H$ values than water from coastal and lower elevations regions [46]. In comparison, the differences in $\delta^{2}H$ values of the Papermate® pens from the three purchase locations are not as large as those observed for Bic® pens (Table 2) and do not exactly mirror the pattern of isotope ratio variation in water from each state. We found that the $\delta^{2}H$ value for the Louisiana Papermate® pens were lower than those for the New Hampshire pens although Louisiana water $\delta^{2}H$ values are expected to be higher than New Hampshire water $\delta^{2}H$ values [46].

Measurement of 7 pens of the same brand demonstrated that ink collected from pens of various (unknown) ages also had different isotope ratios. This variation in isotope ratios of inks through space and time could be due to many factors, including physical processes related to aging, chemical decomposition, or drying within the ink reservoir during transport and storage. The variability could also be due to formulation changes by the ink manufacturer. For example, previous investigations have shown that manufacturers of ballpoint inks can and do change the chemical composition of ink over time due to market influences [18].

4.2. Conclusion and future work

We have demonstrated that there is isotopic variation in inks and there is capacity to distinguish among inks of similar chemical composition using stable isotope ratio analysis. This analytical technique can be applied to examine both ink (ballpoint and gel) and ink on paper. No significant changes to instrumentation are required for the isotope ratio analysis of ink "as is" or on paper, beyond the replacement of the standard water trap in the elemental analyzer with a trap containing a CO_2 absorbent in addition to magnesium perchlorate for measurement of nitrogen isotope ratios of inked paper. Stable isotope ratio analysis thus has the potential to be an extremely useful investigative technique in QDE, especially for pigment-containing gel pen inks that have proven particularly difficult to analyze using the same techniques previously applied to ballpoint pen inks.

As a complement to analysis of inks, the forensic examination of the document substrate (paper) via stable isotope analysis will be useful in questioned document investigations. For example, van Es et al. [47] have published data related to the discrimination of different copy papers using measured stable isotope ratios. Recent work by Jones et al. expanded on this work to more extensively demonstrate the discrimination of paper using carbon stable isotope ratios [37–39]. This suggests that a combination of both ink and paper stable isotope analysis may be more useful for document authentication than analysis of either media or substrate alone.

However, before application of stable isotope analyses of ink in QDE casework is routine, micro-scale sampling and analysis methods must first be developed. The technique as applied here to ink requires large sample sizes (e.g. milligram-size samples) for analysis and material must be collected using destructive sampling techniques. These requirements will limit application of the technique for some types of

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evidentiary material, such as historic documents [48]. As a follow-up to this proof-of-concept demonstration on the use of stable isotope analysis for characterizing ballpoint and gel pen inks, future work should focus on further developing the technique as a viable examination method for the ink found in questioned documents. One promising area of focus is micro-scale sampling and analysis techniques, which would reduce sample size requirements while leaving the sampled documents (and writing) relatively unblemished. Laser ablation is one such micro-scale sampling technique that has been previously used to collect samples of ink on paper for trace element concentration analysis [24,48–50]. An analytical setup recently described by Moran et al. [51] may make it possible to measure the isotope ratio composition of inked paper via laser ablation-isotope ratio mass spectrometry in the future.

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