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Potential Primary Measurement Tool for the Quantification of DNA

Ryan G. Brennan,[†,‡] Savelas A. Rabb,[*,‡] Marcia J. Holden,[§] Michael R. Winchester,[‡] and Gregory C. Turk[‡]

Department of Chemistry, The George Washington University, Washington, DC 20052, Analytical Chemistry Division, and Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg Maryland 20899

An automated sample introduction system, utilizing a demountable direct injection high-efficiency nebulizer (d-DIHEN), is successfully incorporated for the first time with an inductively coupled plasma optical emission spectrometer (ICP-OES) for the measurement of the phosphorus content in acid-digested nucleotides and deoxyribonucleic acid (DNA). With this experimental setup, the solution uptake rate and volume are reduced from 170 to 30 μL min^{-1} and from 10 to 2.4 mL, respectively, thereby reducing the required DNA sample mass for solutions containing 3 μg g^{-1} P from 300 to 72 μg of DNA, in comparison to previous analyses in our laboratory using a glass concentric nebulizer with cyclonic spray chamber arrangement. The use of direct injection also improves P (I) 213.617 nm sensitivity by a factor of 4 on average. A high-performance (HP) methodology in combination with the previous sample introduction system and ICP-OES provides simultaneous, time-correlated internal standardization and drift correction resulting in relative expanded uncertainties (% ***U*****) for the P mass fractions in the range of 0.1−0.4 (95% confidence level) for most of the thymidine 5′-monophosphate (TMP), calf thymus DNA (CTDNA), and plasmid DNA (PLDNA) analyses. The d-DIHEN with HP-ICP-OES methodology allows for the quantification of DNA mass at P mass fractions as low as 0.5 μg g^{-1}, further reducing the required DNA mass to 12 μg, with small uncertainty (≤0.4%). This successful approach will aid in the development and certification of nucleic acid certified reference materials (CRMs), particularly for these samples that are typically limited in volume.**

The accurate mass determination of DNA and other nucleic acids is of critical importance in many clinical diagnoses, forensic, and molecular biological studies. In addition, these measurements could aid in the determination of transgenic material in agricultural and food products. Because many such commodities are imported and exported annually, they are of great economic and trade interest. European nations require labeling of imported crops and prepared foodstuffs that contain greater than 0.9% transgenic material.[1] Such regulations are important to the United States, which is the world's largest exporter of agricultural commodities and prepared foodstuffs with 57.7 million hectares of planted genetically modified (GM) crops (50% of the global area of crops). Many key crops exported from the United States are composed of transgenic material. For example, of the most voluminous U.S. export crops, 64%, 43%, and 24% of the total global planted soybean, cotton, and corn, respectively, are genetically modified as of 2007.[2] For these reasons, the demand has increased for a reliable analysis technique for the development of nucleic acid certified reference materials (CRMs).

The accurate determination of the amount of DNA isolated from a biological material is not trivial. The results of the more common spectroscopic methodologies to quantify DNA preparations, such as absorbance[3] at 260 nm or fluorescent-dye binding,[4,5] can be seriously compromised by impurities in the DNA preparations or state of the DNA itself. These techniques are also not traceable to the SI (International System of Units) because the calibrations cannot currently be performed using calibration standards whose values are traceable to the SI. Such techniques cannot achieve the high accuracy and precision required to produce CRMs for nucleic acid materials. Quantitative, real-time polymerase chain reaction (Q-PCR) is a powerful technique due to its sensitivity, specificity, and applicability;[6] however, it is imperative that an appropriate amount of template DNA be added to the assay so that trace detection is possible. Overloading the template in the reaction can often interfere with the amplification reaction. Therefore, quantification of total DNA is important, prior to conducting Q-PCR studies.

Inductively coupled plasma mass spectrometry (ICPMS) offers higher sensitivity and lower detection limits compared to inductively coupled plasma optical emission spectroscopy (ICP-OES); however, ^{31}P measurements by ICPMS suffer from interferences ($^{14}N^{16}O^{1}H^{+}$ and $^{15}N^{16}O^{+}$) and incomplete ionization (P has a

* To whom correspondence should be addressed. Phone: 301-975-8504. Fax: 301-869-0413. E-mail: savelas.rabb@nist.gov.
† The George Washington University.
‡ Analytical Chemistry Division, National Institute of Standards and Technology.
§ Biochemical Science Division, National Institute of Standards and Technology.

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high first ionization potential ~10.5 eV).[7] The use of a collision cell, reaction cell, or sector field (SF) in ICPMS studies has been observed to successfully remove these interferences.[8,9] However, the ICP-SFMS can typically provide an order of magnitude greater sensitivity than the collision cell and reaction cell at a mass resolution of 4000.[10] Bruchert and Bettmer[8] achieved precisions lower than 3% for separated DNA fragments with online coupling of gel electrophoresis (GE) and ICP-SFMS. However, the accuracy of their method could not be reported due to the lack of CRMs for DNA mass. Recent work to overcome such issues utilizes element tagging or nanoparticle labeling of nucleic acids and proteins, where a metallic element or metal nanoparticle is measured instead of ^{31}P.[11−14] This concept was established by Tanner and co-workers,[11,12] who reported detection limits of 0.1−0.5 ng mL^{-1} for a target protein with a linear response to protein concentrations over 3 orders of magnitude. More recently, Kerr and Sharp[15] labeled biotinylated DNA with a gold nanoparticle containing a streptavidin functionality. They separated and determined the labeled DNA by high-performance liquid chromatography−ICPMS (HPLC−ICPMS). This resulted in a detection limit that would be equivalent to a ^{31}P detection limit of 500 pg L^{-1}, had ^{31}P been measured. With a labeled metal nanoparticle, the problems associated with ^{31}P detection in ICPMS are eliminated. However, the accuracy of the measurement and enhancement in the analytical sensitivity is dependent on the determination of how many gold atoms have been bound and their locality, because the ^{197}Au signal is being measured and not ^{31}P. This area of research is still developing and will be important for quantitative analyses of DNA and nucleic acid samples that require very low detection limits. Patel et al.[16] introduced a potential application of isotopic labeling of peptides with detection by HPLC−ICPMS. The $^{151}Eu/^{153}Eu$ isotope ratio was utilized to calculate the original peptide ratio. Peptides were labeled with natural and isotopically enriched Eu^{3+}, which enabled the detection and relative quantification of peptides within 5.2% of the known ratio.

Some other new, alternative approaches to quantify DNA include the use of laser-induced breakdown spectroscopy (LIBS) and an electrochemiluminescence biomarker barcode method. The approach by Le Meur et al.[17] allows for quantification of nucleic acids on a support without labeling using LIBS. This technique lacks sensitivity in comparison to most, but it provides the advantage of avoiding problems associated with attaching fluorophores into nucleic acids. Zhu et al.[18] introduced a PCR-free method to quantitatively detect genetically modified organisms (GMOs) by use of a biomarker bar code method, eliminating the need for PCR amplification which can be time-consuming and often leads to false identification. The authors report high specificity and sensitivity with the ability to detect GMOs from raw material without purification.

At the National Institute of Standards and Technology (NIST) in collaboration with The George Washington University, a methodology that can be used to provide accurate measurements of DNA and nucleic acid mass that are traceable to the SI is being developed with the long-term goal of providing a CRM for DNA mass. Currently, the ICP-OES approach developed at NIST, referred to as high-performance ICP-OES (HP-ICP-OES)[19,20] incorporating a ratio-based technique with drift correction,[21] has been utilized for the measurement of phosphorus content of acid-digested nucleotides and DNA. The HP-ICP-OES measurement of phosphorus provides a highly accurate quantification of mass for both nucleotide monophosphate and DNA with relative uncertainties less than 0.1% (95% confidence level). However, this approach also requires a significant sample size (10 mL of a 3 μg g^{-1} P solution or 300 μg of DNA), restricting its usefulness for the routine quantification of DNA.[22] A similar observation was reported by Yang et al.[23] in their accurate quantification of oligonucleotides using ICP-OES, consuming 1.7 mg of sample per measurement.

For this reason, a demountable, direct injection high-efficiency nebulizer (d-DIHEN)[24,25] was utilized to facilitate the introduction of samples into the ICP-OES at low sample uptake rates, limiting sample consumption. For the d-DIHEN to work successfully with the HP-ICP-OES methodology, the nebulizer had to aspirate the sample in a "pulse-free" mode and function well with an autosampler. Pulse-free aspiration was achieved using a special pump head that created opposing pulse patterns in dual sample lines that canceled when the solution lines were combined. A microswitch was constructed to halt the peristaltic pump as the sample probe moved from vial to vial, thereby eliminating the introduction of air bubbles that can destabilize the plasma. This is the first report of the d-DIHEN operating in a completely automated sampling mode. With the implementation of the HP-ICP-OES method[22] in combination with the d-DIHEN, the solution uptake rate can be reduced from 170 to 30 μL min^{-1}, and the sample size can be reduced from 10 to 2.4 mL. Consequently with the increase in sensitivity using the d-DIHEN, the required DNA mass for digestion can be reduced from 300 μg for a 3 μg g^{-1} P solution to 12 μg for a 0.5 μg g^{-1} P solution, while achieving the majority

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of relative measurement uncertainties on the order of 0.4% or less.

EXPERIMENTAL SECTION

Preparation of Nucleotides and Nucleic Acids. The nucleotide monophosphate, genomic DNA, and plasmid DNA used in this study were thymidine 5′-monophosphate (TMP), DNA sodium salt from calf thymus (CTDNA), and a plasmid (pPCR Script Amp, Stratagene) propagated in *Escherichia coli* (PLDNA), respectively. Both the TMP and CTDNA were obtained from Sigma Chemical Co. (T-9758 and D1501). (Identification of commercial products in this paper was done in order to specify the experimental procedure. In no case does this imply endorsement or recommendation by the National Institute of Standards and Technology.) Samples were stored in 1 mM Tris buffer (pH 8) and 5% HCl (v/v). The details of preparation for these samples are discussed elsewhere.[22]

Phosphorus Measurements: ICP-OES. In prior efforts, HP-ICP-OES[19−21] was used to determine the phosphorus mass fraction with an internal standard spike of Ge to help correct for high-frequency noise and a drift correction procedure to correct for low-frequency noise. Any observed difference between the sample and calibration standard is used to compute the phosphorus mass fraction in the sample. This calculation is described by the following equation:[21]

$$\text{P mass fraction } (\mu g\ g^{-1}) = \left(\frac{\left(\frac{I_P}{I_{Ge}}\right)_{Unknown}}{\left(\frac{I_P}{I_{Ge}}\right)_{Calibrant}}\right)\left(\frac{\left(\frac{m_P}{g_{Ge}}\right)_{Calibrant}}{\left(\frac{m_P}{g_{Ge}}\right)_{Unknown}}\right) \quad (1)$$

where I is the signal intensity and m is the mass of the P or Ge in the calibrant and unknown solutions. Typically, the analyte mass fraction in the calibrants very closely approximates the analyte mass fraction in the unknown, resembling a single-point calibration. The calibration relationship between the signal intensity ratio and the mass ratio is linear with an intercept of zero as long as the blank and background are appropriately corrected for in the signal intensity ratio. The HP-ICP-OES method was utilized due to its ability to provide elemental determinations with relative expanded uncertainties (% U) that are better than a few parts per thousand (95% confidence level).[19−21]

An Optima 5300 DV ICP-OES instrument (Perkin-Elmer, Inc., Shelton, CT) was used for these determinations with the operating conditions listed in Table 1. The nebulizer position and gas flow rate, along with the position of the solution capillary, were optimized to provide the best analytical performance while maintaining robust plasma conditions. In a previous DIHEN ICP-OES study,[26] the nebulizer gas flow rate was optimized at a similar value to that of the d-DIHEN when the maximum rf power was used to statistically determine the gas flow rate that offered the most robust plasma. Using the Mg (II) 280.270 nm/Mg (I) 285.213 nm intensity ratio, the most robust plasma was formed at a ratio of 6.6 and 9.2 for the DIHEN and d-DIHEN, respectively. These values have been corrected for the differing Echelle grating diffraction efficiencies at the two wavelengths by multiplying the observed ratio by 1.85.[27] The d-DIHEN[24,25] provides an adjustable solution capillary, permitting optimization of aerosol properties to generate superior analytical performance, to operate more efficiently at reduced solution flow rates. The more robust plasma conditions observed within this work can be attributed to the reduced critical dimensions of the d-DIHEN, which results in a much finer aerosol and reduces the solvent load on the plasma. The selection of the P (I) 213.617 nm and Ge (I) 265.118 nm lines was due to similar excitation energies and the observed intensity ratio precision (% RSD). The ratio RSDs (repeatability) ranged from 1.5% to 0.2% (based upon five replicates) for 0.5−3.0 $\mu g\ g^{-1}$ P, respectively. The best RSDs are achieved for 3 $\mu g\ g^{-1}$ P (0.2−0.4%) due to the larger signal intensity (larger S/N) observed in comparison to 0.5 $\mu g\ g^{-1}$ P. A segmented-array, charge-coupled device detector was used for signal integration. The integration parameters were set manually for each P/Ge mass fraction ratio to provide simultaneous acquisition of the signal. The intensity of Ge (I) 265.118 nm was generally within a factor of 2 of the intensity for P (I) 213.617 nm to allow for the best correlations. On-chip integration times were selected to be 1−2 s to provide some improvement in ratio precision of the P/Ge intensity ratio.

To reduce solution uptake rate and sample size, a d-DIHEN was utilized with critical dimensions (Table 2) successful in a previous work with similar challenges.[28] A CETAC ASX-100 microautosampler (CETAC Technologies, Omaha, NE) was used with a Gilson Minipuls-3 peristaltic pump (Gilson Inc., Middletown, WI) for microsample automation and solution delivery. To improve precision, a Spetec Perimax 16/2 Antipuls pump head was installed onto the pump to deliver a "pulse-free" solution flow rate (Spetec GmbH, Erding, Germany). The pulsation that is commonly observed with the use of a peristaltic pump is eliminated through

Table 1. Operating Conditions for Argon ICP-OES

ICP-OES system	Perkin-Elmer Optima 5300 DV
power (kW)	1.5
plasma gas (L min^{-1})	15
auxiliary gas (L min^{-1})	0.5
nebulizer gas (L min^{-1})	0.13
viewing	axial
sample uptake (μL min^{-1})	30
analyte wavelength (nm)	P I 213.617
reference wavelength (nm)	Ge I 265.118
on-chip integration time (s)	1.024 or 2.048
total read time (s)	8.192
nebulizer position (mm) (with respect to intermediate torch)	−4
solution capillary position (μm) (with respect to nebulizer gas nozzle)	±10

Table 2. Critical Dimensions for d-DIHEN

solution capillary i.d. (μm)	75
capillary wall thickness (μm)	12
solution capillary o.d. (μm)	138
gas nozzle orifice i.d. (μm)	150

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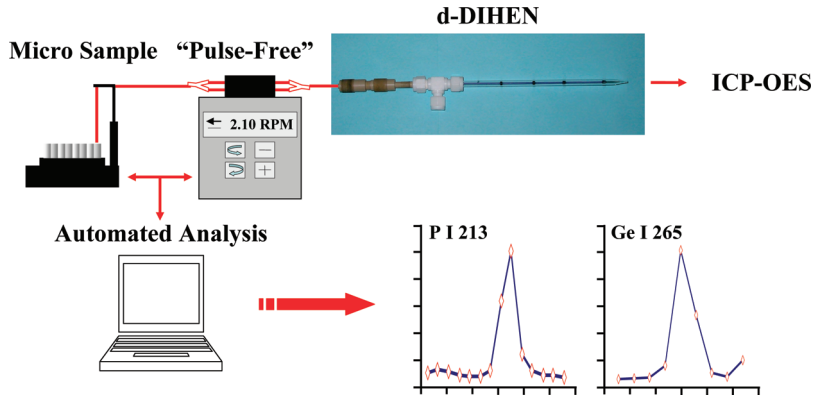

Figure 1. Schematic of typical setup for coupling the d-DIHEN to a Perkin-Elmer Optima 5300 DV ICP-OES for automated, pulse-free analysis.

the use of double-stranded pump tubing and the phase displaced roller head of the Spetec Perimax 16/2. The solution uptake is split in two by a y-connecter (P/N: P-512, Upchurch Scientific, Oak Harbor, WA) to construct the double-stranded pump tubing, allowing each solution line to pass over separate sets of rollers that are offset from each other. The wave peak in one solution line coincides with the wave trough in the other line so that the peaks and troughs cancel each other out. This action provides a "pulse-free" flow as the two solution lines are recombined together with another y-connecter downstream from the roller head. A schematic of the experimental setup is shown in Figure 1.

Since the introduction of the DIHEN series,[24,25,29–31] no attempts to automate sample delivery with these nebulizers have been reported. One of the main disadvantages of automation with these nebulizers is the introduction of air bubbles in the solution line that could extinguish the plasma in most ICP instruments. Since an external peristaltic pump was used to provide pulse-free aspiration, a microswitch was fabricated from copper foil, to control the operation of the peristaltic pump via the positioning of the sample probe on the microautosampler. The switch was constructed by connecting two wires from a copper contact on the sample probe to the peristaltic pump start/stop contacts (pins 3 and 4). The switch could then be closed (pump started) when the autosampler probe was lowered and opened (pump stopped) when the probe was raised (Figure 2). The microswitch prevents an air bubble from being introduced into the solution line when the microautosampler probe moves from one sample vial to the next. Thus for the first time, an automated procedure was developed for use with the d-DIHEN, without plasma shutdowns.

For all analyses, four solutions were prepared gravimetrically from stock solutions for both the calibration standards and nucleic acid samples (TMP, CTDNA, and PLDNA). The TMP and CTDNA sample stock solutions, not limited by sample volume like PLDNA, were each subdivided into two solutions (TMP (1), TMP (2), CTDNA (1), and CTDNA (2)) for a means of comparison. All samples were diluted and analyzed at three different P mass fractions (0.5, 1.0, and 3.0 μg g^{-1}). The calibrants were matched very closely in P mass fraction and matrix to that of the samples. The sample and calibrant solutions were also spiked

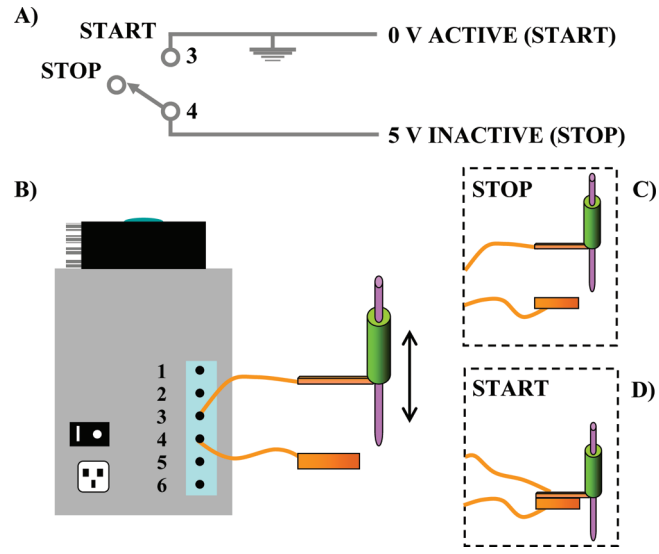

Figure 2. Schematic and operation of "microswitch" to allow for automated analysis with the d-DIHEN: (A) electrical control diagram, (B) Gilson Minipuls-3 peristaltic pump with 6-pin barrier strip, (C) microswitch with autosampler probe in "stop" position, and (D) microswitch with autosampler probe in "start" position.

with a Ge solution with a mass fraction of 10 μg g^{-1} to obtain a final Ge mass fraction of 0.5−1.0 μg g^{-1} (depending on the P mass fraction analyzed). Phosphorus calibration standards were prepared from NIST SRM 194 (ammonium dihydrogen phosphate) with the internal standard solution from a 10 000 μg mL^{-1} Ge CRM solution (Inorganic Ventures, Lakewood, NJ). A single determination consisted of five repeat measurements (random order), requiring approximately 6−9 h to complete each analysis. Determinations at 0.5, 1.0, and 3.0 μg g^{-1} P were each analyzed twice (except for PLDNA at 3 μg g^{-1} P) to calculate the average P mass fraction for a given sample in the original stock. All solutions were prepared in analytical grade 2% HNO_3 (v/v) and a 1 mM Tris buffer.

As a means of method validation, the P content of TMP was measured both by HP-ICP-OES and gravimetric preparation (i.e., measurement on a balance). Subsequently, the TMP was further utilized as a control in the analyses of the CTDNA and PLDNA.

Measurement Uncertainties. All uncertainties shown for the data consist of expanded uncertainties expressed at the 95% level of confidence and are calculated according to the principles of the ISO Guide to the Expression of Uncertainty in Measurement

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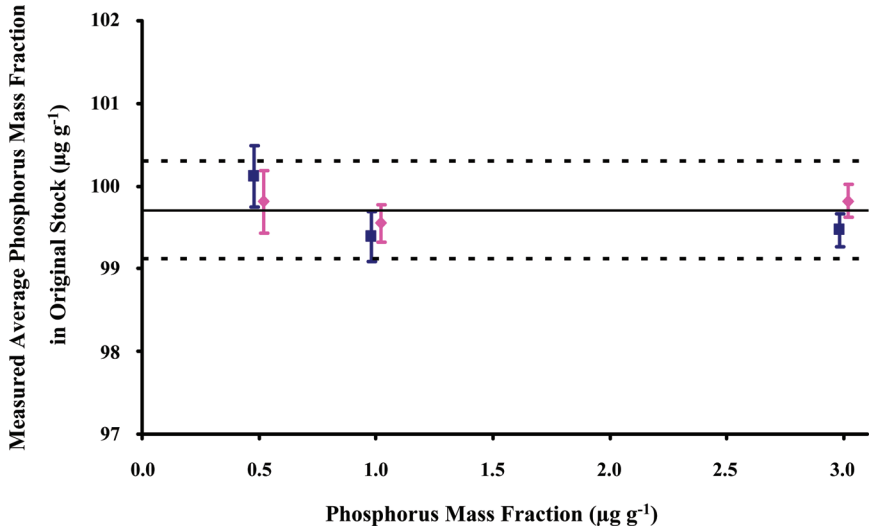

Figure 3. ICP-OES measurements of the average P content of the nucleotide TMP compared to the gravimetric value. The TMP samples were analyzed at three different P mass fractions, 0.5, 1.0, and 3.0 μg g^{-1} P, in two trials: square—TMP (1) and diamond—TMP (2). Each trial represents the average of two experiments. Please note that the data points are offset from the *x*-axis value only to allow the error bars from each trial to be observed. The relative expanded uncertainties (% *U*) for all the TMP analyses are represented by the error bars (95% confidence level). The solid line represents the P gravimetric value, with the dashed lines representing the measurement uncertainty of the gravimetric value.

(GUM).[32] Expanded uncertainties were determined for ICP-OES phosphorus measurements as well as the gravimetric preparations of TMP by using the following equations:

$$u_c = \sqrt{u_1^2 + u_2^2 + u_3^2 + \ldots} \quad (2)$$

$$U = ku_c \quad (3)$$

$$\%U = \frac{U}{x} \times 100 \quad (4)$$

where u_i represents the individual component of uncertainty, u_c is the combined uncertainty, k is the expansion factor based on the Student's t for the chosen level of confidence, U is the expanded uncertainty, and x is the observed measurement of P or TMP mass. Propagated components of uncertainty include observed measurement repeatability, observed variability in the determination of ICP-OES sensitivity, and uncertainties in the known values for the calibration standards. Uncertainties in the gravimetric preparation of TMP include those associated with the mass of TMP, the mass of the 5% HCl solution, Karl Fischer determination of the water content of TMP, and possible undetected TMP impurities.[22] The absorbance measurements for the plasmid and CTDNA were made on a Beckman DU-650 spectrophotometer using a quartz cuvette. The expanded uncertainties are based on the measured standard uncertainty where the coverage factor $k = 4.3$.

RESULTS AND DISCUSSION

The direct injection high-efficiency nebulizer (DIHEN) and d-DIHEN were introduced in 1998 and 2003, respectively, by Montaser and co-workers.[24,25,29,30] The DIHEN and d-DIHEN, compared to the conventional nebulizer−spray chamber arrangement, offer the user (1) 100% sample transport efficiency, (2) a low internal dead volume and thus rapid response times and reduced memory effects, (3) improved precision by eliminating noise sources attributed to the spray chamber; and (4) similar or improved detection limits and sensitivity when operated at microliters per minute sample uptake rates.

Measurements of the average P content of the nucleotide TMP, one of the four common base components of DNA, are pictured in Figure 3. The TMP samples were analyzed at three different P mass fractions, 0.5, 1.0, and 3.0 μg g^{-1} P to determine the effects on accuracy and precision as the P mass fraction was varied. The ICP-OES measured average P mass fraction in the TMP original stock solution is virtually the same for both TMP (1) and TMP (2) at each mass fraction, thus providing method validation. The relative expanded uncertainties for all the TMP analyses ranged from 0.20% to 0.38%. In comparison to the results using our previous sample introduction system, the expanded uncertainties are slightly larger for the d-DIHEN. Also, the expanded uncertainties increase as the sample P mass fractions decrease. However, these increases are more than acceptable for accurate determinations of P in this material. As a result, limited samples, previously analyzed near 3 μg g^{-1} P, can be increased through dilution (a factor of ~10 in this case). Finally, the differences between the d-DIHEN-HP-ICP-OES values for the P mass fraction and those from the gravimetric measurement ranged from 0.11% to 0.42%. These results are comparable to or better than previous results[22] (without d-DIHEN technology) demonstrating a consistent correlation between ICP-OES measurements, regardless of the sample introduction system, and gravimetric measurements.

Determinations of the average mass fractions of phosphorus in CTDNA and PLDNA are shown in Figures 4 and 5. Three different P mass fraction levels are also investigated for these samples. The ICP-OES measured P mass fractions for all the DNA analyses are in very good agreement with each other. This

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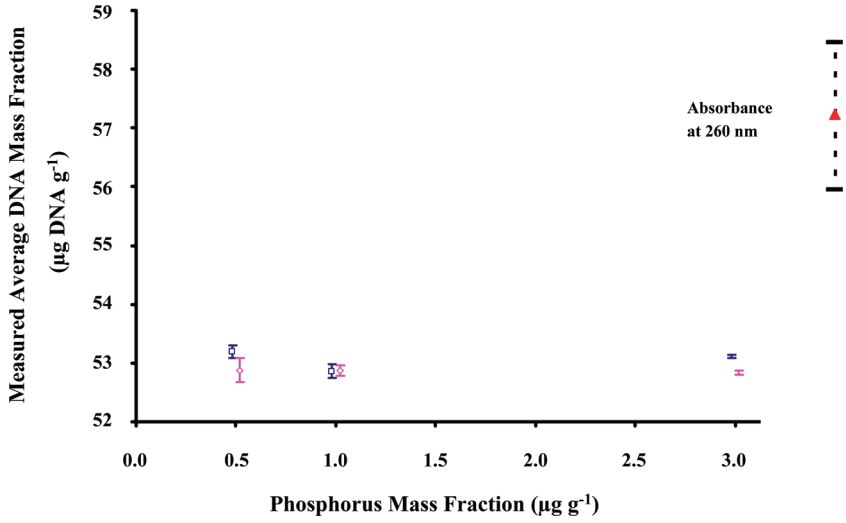

Figure 4. ICP-OES measurements of the average DNA mass fraction of the prepared calf thymus DNA (CTDNA) samples compared to the absorbance measurement at 260 nm. The CTDNA samples were analyzed at three different P mass fractions, 0.5, 1.0, and 3.0 μg g^{-1} P, in two trials: square—CTDNA (1) and diamond—CTDNA (2). Each trial represents the average of two experiments. Please note that the data points are offset from the *x*-axis value only to allow the error bars from each trial to be observed. The relative expanded uncertainties (% *U*) for all the CTDNA analyses are represented by the error bars (95% confidence level). The triangle represents the DNA mass fraction determined by the absorbance measurement at 260 nm, with the dashed error bar representing the measurement uncertainty.

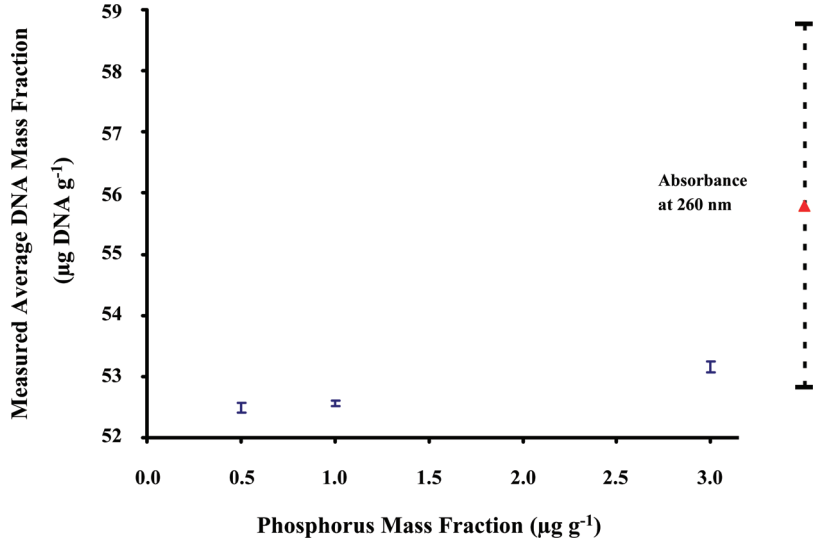

Figure 5. ICP-OES measurements of the average DNA mass fraction of the prepared plasmid DNA (PLDNA) samples compared to the absorbance measurement at 260 nm. The PLDNA samples were analyzed at three different P mass fractions, 0.5, 1.0, and 3.0 μg g^{-1} P. Each value represents the average of two experiments, except for 3.0 μg g^{-1}, which was a single experiment. The relative expanded uncertainties (% *U*) for all the PLDNA analyses are represented by the error bars (95% confidence level). The triangle represents the DNA mass fraction determined by the absorbance measurement at 260 nm, with the dashed error bar representing the measurement uncertainty.

suggests that matrix effects are likely insignificant as the least concentrated and most concentrated samples still returned a similar P mass fraction in the stock solution. Also, good ratio precision of the P/Ge intensity ratio (0.1−2.0%) for the analysis measurements imply that the digestion of these different types of DNA samples are complete as fragments would have resulted in erratic signal intensities. The calculated DNA mass showed reasonable relative expanded uncertainties overall (0.12−0.42%) with 0.75% being the highest for the analysis of one of the sets of CTDNA samples. Reduction of the required sample size with the use of the d-DIHEN was particularly important for the analysis of the PLDNA that was previously limited to a single experiment per sample.

The ICP-OES measurements are consistently 8% and 6% lower than the absorbance measurement of DNA mass for the CTDNA and PLDNA, respectively. Previously, a negative bias with respect to absorbance was also observed for CTDNA and PLDNA, 8% and 10%, respectively, without using the d-DIHEN. A negative bias (−) is preferred because a positive bias of the DNA mass fraction could arise from impurities (phosphorus-containing molecules) present in the samples or contamination during the sample analysis, thereby overestimating the mass fraction of CTDNA and PLDNA. English et al.[33] compared ICP-OES measurements to four other quantification methods for DNA mass and reported biases of −8% to 38%. The improvement in our measurements can be

(33) English, C. A.; Merson, S.; Keer, J. T. *Anal. Chem.* **2006**, *78*, 4630–4633.

attributed to the use of the absorbance value of alkali denatured DNA and the use of the extinction coefficient for single-stranded DNA versus the extinction coefficient for double-stranded DNA.[34] The smaller uncertainty observed in the absorbance measurement of the CTDNA versus that of the PLDNA (Figures 4 and 5) can be the result of sample preparation. Variability in the dilution of the PLDNA samples with NaOH could have occurred. The issue with bias is not yet solved with respect to differences in DNA mass determinations conducted by absorbance and ICP-OES and requires further investigation. However, the use of a method such as HP-ICP-OES in the development of CRMs for DNA mass may lead to an increase in the accuracy of current methods. A more precise and accurate quantification of DNA may be of value to revisit the issue of the extinction coefficient for DNA that was determined in 1948 by Chargaff and Zamenhof.[35]

The results of the analysis of TMP, CTDNA, and PLDNA are reproducible with reasonable expanded uncertainties regardless of using our previous setup (i.e., a cyclonic spray chamber and concentric nebulizer) or the d-DIHEN. We have devised a way for the analytical community to employ an automated process for methods necessitating small sample size and reduced solution flow rates, while being able to utilize all the aforementioned benefits of the d-DIHEN. The microswitch and pulse-free flow create a more user-friendly d-DIHEN with no plasma shutdowns and increase the potential for the d-DIHEN to be used more widely. The reduced critical dimensions allow for the highest reported Mg (II) 280.270 nm/Mg (I) 285.213 nm intensity ratio (9.2) for a d-DIHEN with an argon ICP-OES. With this setup (Figure 1), the solution uptake rate was reduced from 170 to 30 μL min^{-1} and the required sample size was reduced from 10 to 2.4 mL. Additionally, the 4-fold increase in the P (I) 213.617 nm sensitivity allowed for a decrease in the P mass fraction (from 3 to 0.5 μg g^{-1}) analyzed and DNA mass consumed (from 300 to 12 μg) compared to a glass concentric nebulizer with cyclonic spray chamber arrangement.

In the future, the analysis of the TMP, CTDNA, and PLDNA without the use of the Tris buffer will be investigated. The Tris buffer helps to keep the nucleotide and DNA stable in the solution prior to analysis; however, a factor of 2 reduction in sensitivity is observed, compared to samples using only a 2% HNO_3 (v/v) matrix. During the acid digestion of the DNA samples, the most abundant element in the samples, carbon, will likely convert to carbonic acid and subsequently evolve as carbon dioxide. However, the Na contribution from the sodium acetate used in the preparation of the DNA could be a factor in the signal suppression. More work will be conducted in this area at a later date. Yet, even with this reduction, the current setup with the use of the d-DIHEN offers a factor of 40 improvement compared to the previous sample introduction system, considering the solution flow rate, material required, and the increase in P (I) 213.617 nm sensitivity. Without the Tris buffer, the current technique could provide accurate quantification of phosphorus with small uncertainty at mass fractions potentially as low as 0.1 μg g^{-1} (2.4 μg DNA).

CONCLUSIONS

An automated sample analysis system, utilizing the d-DIHEN, for ICP-OES was successfully incorporated for the accurate measurement of the phosphorus content of acid-digested nucleotides and DNA with small uncertainty. The solution uptake rate was reduced, as observed in our previous analyses, from 170 to 30 μL min^{-1} and the required sample size was reduced from 10 to 2.4 mL, while providing relative expanded uncertainties (% *U*) in the range of 0.1−0.4 (95% confidence interval) for most of the TMP, CTDNA, and PLDNA analyses. The use of direct injection improves P (I) 213.617 nm sensitivity by a factor of 4 on average compared to a glass concentric nebulizer with cyclonic spray chamber arrangement, permitting the consumption of 12 μg of DNA analyzing a 0.5 μg g^{-1} P solution. This successful method will aid in the development of CRMs with nucleic acid content, particularly for samples that are typically limited in volume.

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