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COMMUNICATION

Towards the Use of Inductively Coupled Plasma Optical Emission Spectrometry for the Elemental Analysis of Organic Compounds such as Glucosamine

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Inductively coupled plasma optical emission spectrometry with a long demountable torch (whose outer tube is 2.5-cm longer than a standard torch) and a conventional pneumatic nebulisation system was applied to the determination of C, H and O concentrations from an organic compound dissolved in water. The sample solution was simply aspirated directly into the plasma without any sample pre-treatment (such as desolvation or degassing). The long torch was required to significantly decrease the background arising from entrained air. Good linearity was obtained for C, N, H and O upon calibration with standard solutions prepared from ultrapure tris(hydroxymethyl)aminomethane. A weighed blank correction was applied to compensate for the contribution from water, especially in the cases of H and O. Under these conditions, the detection limits for C, N, H and O were, respectively, 0.2, 50, 1000 and 2000 µmol of analyte per g of solution. Accurate concentrations, according to a Student`s t test at the 95% confidence level, were measured for C, H and O in a solution of D-glucosamine hydrochloride. No internal standardisation was necessary. However, the N concentration was biased high, irrespectively of the N emission line used (from 149.262 to 593.178 nm), which rules out spectroscopic interference and will be further investigated. Nonetheless, the possibility of accurately determining C, H and O simultaneously with trace elements makes the approach quite promising. It is much simpler than alternate methods that require derivatisation of the compound prior to detection.

Introduction

Glucosamine is an essential amino-monosaccharide that is synthesised from glucose in vivo, so naturally occurs in both animals and humans in connective tissues as well as in gastrointestinal mucosal membranes, where it plays important roles in sustaining and restoring the function of articular cartilage.[1] Because in vivo synthesis of glucosamine may gradually decrease with aging, leading to osteoarthritis and degenerative joint disease, a number of commercially-available nutritional supplements (also called nutraceuticals) containing glucosamine (usually in the form of glucosamine sulphate or glucosamine hydrochloride) have become available. Glucosamine is also used to treat arthritis (more specifically, osteoarthritis) and has been determined to have a low toxicity.

However, because glucosamine is usually obtained from acid hydrolysis of chitin (a polymer chain of N-acetyl glucosamine), which is the main component of the cell walls of fungi, the exoskeletons of arthropods and insects, as well as the beaks of cephalopods, from which it is extracted,[2] and that chitin can bind elements such as As to the point that it can be used to remove them from contaminated drinking water and groundwater,[3,4] there is a large risk of contamination of the resulting glucosamine. For instance, 9 mg/g As was determined in a supplement whose major component was glucosamine hydrochloride.[5] Yet, because glucosamine is a dietary supplement, not a medication, it is not subject to regulatory quality and purity control.[6,7] This leads to great variability in nutraceutical product content and composition,[7] including glucosamine content. For example, the amount of glucosamine in commercially-available supplements was reported to vary from 41 to 108% of the free base weight stated on the label.[6] To further complicate matters, glucosamine and its derivatives are conventionally determined using high performance liquid chromatography (HPLC) coupled with ultraviolet-visible spectrophotometry,[8] fluorescence[1] or electrospray ionization mass spectrometry,[9] which requires derivatization of the analyte prior to detection. This is not only time-consuming, but may also result in contamination or analyte loss.

The identification of organic compounds by atomic emission spectrometry (AES) through determination of their empirical formula has so far only been carried out by gas chromatography coupled to a microwave induced plasma (MIP).[10-18] However, this approach has a number of drawbacks. It is only applicable to relatively volatile thermally-stable compounds, thereby requiring derivatisation of non-volatile analytes, which significantly increases the analysis time and sources of error. The MIP-AES instrument response is also compound-dependent,[17-18] which further complicates the analysis. Indeed, unless they elute close

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to the analyte,[10] standards with a similar structure may be required for calibration,[11] which are not always available. Alternatively, a complementary detector such as mass spectrometry (MS) must be used to confirm the identity of analytes.[10,12,13]

On the other hand, inductively coupled plasma optical emission spectrometry (ICP-OES) is commonly used for the simultaneous determination of numerous major, minor and trace elements in solution, while the lower detection limit of ICP-MS makes the latter a prerequisite for ultra-trace multi-element analysis.[19] However, because ICP-OES involves the measurement of emitted light, which is a completely passive process, it is inherently more robust than ICP-MS where ions must be physically extracted from the ICP, and can thus be applied to the analysis of solutions containing greater dissolved solid concentrations.[19] As a result, ICP-OES is more readily applied to the analysis of complex matrices, including organic ones. It has been used for the determination of essential and toxic elements in dietary supplements.[7]

However, ICP-OES has never been applied to the determination of organic compounds. Yet, the simultaneous determination of metals and non-metals, in particular C, O and H that compose an organic compound, should be possible, in turn allowing the simultaneous determination of both toxic elements and glucosamine without having to perform a separate analysis using an HPLC-based method mentioned earlier. As the determination of metals by ICP-OES is well established, this paper focuses on the development of a quick and easy ICP-OES method to determine C, H and O in organic compounds, exemplified by glucosamine. To the best knowledge of the authors, this is the first report on the application of ICP-OES to the accurate determination of hydrogen and oxygen in aqueous solutions of organic compounds.

Experimental

Instrumentation

Method development was done using a lateral-view ARCOS ICP-OES instrument (SPECTRO Analytical Instruments, Kleve, Germany) with Smart Analyzer Vision (version 3.01.0752) software. The standard torch was replaced with a demountable torch (Glass Expansion, Pocasset, MA, USA) having a 2.5 cm longer outer tube than the regular torch so as to ensure that the plasma observation window (through which emitted light goes into the polychromator) would see the plasma through the quartz torch. Such arrangement reduced air entrainment in that region of the plasma, thereby decreasing the emission background for N.

A multimode sample introduction system (Burgener Research Inc., Mississauga, Ontario, Canada) operated in pneumatic nebulisation mode with a Burgener Mira-mist parallel path nebulizer (Burgener Research Inc., Mississauga, Ontario, Canada) was used for most of the work. A CETAC U-6000 AT+ ultrasonic nebuliser with heater/condenser (CETAC Technologies, Omaha, Nebraska, USA) was also used for some tests. In both cases, the sample uptake rate was 2.0 mL/min. Torch position was optimised along with other ICP-OES instrumental operating conditions while monitoring the N 174.273 nm atomic emission line and continuously nebulising a tris(hydroxymethyl)aminomethane (Tris) solution prepared by dissolving 173 g of Tris in 1 L of DDW. Instrumental conditions maximizing the signal-to-background ratio were selected, which are summarised in Table 1.

Chemicals and reagents

All solutions were prepared by weight in doubly deionised water (DDW) from an Arium Pro DI system (from Sartorius Stedim Biotech, Mississauga, ON, Canada). All bottles were cleaned by filling them with a 10% solution of nitric acid (ACS Plus, Fischer Scientific, Ottawa, ON, Canada), leaving them overnight (at the least) to soak, and then thoroughly rinsing them with DDW before usage. D-glucosamine hydrochloride (>99% purity, crystalline form, Sigma-Aldrich Canada, Oakville, ON, Canada) and ultrapure grade (≥99.9%) Tris (Aldrich, St. Louis, MO, USA) were dried to constant weight in an oven at 60ºC prior to dissolution. Their molecular weights are 251.632 and 121.14 g mol^{-1} respectively. As soon as a solution was prepared, it was transferred to a container that was immediately capped to minimize air dissolution. Four standard solutions were prepared by dissolving 2 to 8 g Tris in 25 g DDW and then used for external calibration along with a DDW blank. A solution prepared with 1 g of D-glucosamine hydrochloride in 10 g DDW was used as test solution.

Data processing

Raw emission signals, readily obtainable from the instrument through a simple copy/paste if the file and method names are known for the desired set of data, were used in calculations instead of the automatic background-corrected ones supplied by the instrument. All data processing was done in Excel. Because the amount of water (which was less than 100%) varied between samples and was different than for the blank (which contained 100% water), direct blank subtraction would over-correct the contribution of water on the H and O signals. Hence, a weighed blank correction had to be done using:

$$\text{analyte signal} - \frac{\text{mass of water}}{\text{total mass of solution}} \times \text{blank signal}$$

While the above correction was negligible for C, it was very large for O and H and non-negligible for N. So, it was systematically applied.

Results and Discussion

Optimization of experimental conditions

Attempts were made to determine the concentrations of C, H, O and N using a standard ICP torch. However, with the exception

Table 1: Instrumental operating conditions for lateral view SPECTRO ARCOS ICP-OES instrument

RF power (W)	1400
Nebuliser gas flow rate (L/min)	0.55
Auxiliary gas flow rate (L/min)	3.00
Plama gas flow rate (L/min)	13.00
Plasma observation height (mm above load coil)	11.00
Sample uptake rate (mL/min)	2.00
Atomic emission lines (nm)	C 247.856; N 149.272; H 397.007; O 130.485

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of C, the background arising from ambient air was too high. For instance, using an ultrasonic nebuliser with heater/condenser to remove as much of the water as possible had no effect on the N background emission. To further confirm that the problem was arising from air entrainment, ultra high purity argon gas was used to degas all solutions before their introduction into the ICP, which still resulted in inaccurate and imprecise results. Finally, the procedure of Jaber et al.,[20] who performed the quantitative determination of ammonium and organic-bound nitrogen in aqueous and solid samples by ICP-OES through generation of ammonia vapour, reduced the N background signal relative to that arising from samples and standard solutions. Although this improved precision, inaccurate results still resulted.

Only with a long demountable torch could the N emission background be substantially decreased. For example, the average blank N 174.525 nm signal with a standard torch was 135800 ± 1900 counts/s, whereas it was reduced to 3262 ± 10 counts/s with the long torch i.e. a 42-fold reduction in intensity. Operating conditions were optimized while monitoring the N 174.273 nm atomic emission line to maximize the signal-to-background ratio, which yielded the conditions that were used for the remainder of this work and are summarised in Table 1.

Analytical figures of merit

Under those conditions, the analytical figures of merit summarised in Table 2 were obtained from a calibration curve with Tris standard solutions prepared in DDW and a DDW blank. The N background at 174.273 nm is similar to that reported by Nham [21] for the determination of N in fertilizers using an extended ICP torch. As can been seen from the square of the correlation coefficient, good linearity was obtained for C, N, O and H emission intensities as a function of analyte concentration. No internal standardisation was necessary. The detection limit was calculated as 3 times the standard deviation of the signal from the blank divided by sensitivity (i.e. the slope of the calibration curve). In any case, the high background arising from the continuous nebulisation of aqueous solutions understandably translated into poorer detection limits for O and H than for N and especially C. However, this higher detection limit may be compensated by the fact that several O and especially H atoms are often present in organic molecules (for example, the empirical formulae of Tris and D-glucosamine hydrochloride are $C_4H_{11}O_3N$ and $C_6H_{14}O_5NCl$ respectively).

Application to the analysis of D-glucosamine hydrochloride

The measured concentrations for D-glucosamine hydrochloride obtained by external calibration with standard solutions of Tris are compared to the expected values in Table 3. With the exception of N, the measured concentrations are clearly in agreement with the expected values according to a Student's t test at the 95% confidence level. Similar results could be obtained with other emission lines. For example, using the H 410.174 nm line gave 0.0700 ± 0.0082 mol H per g of glucosamine. However the detection limit with this line was twice that obtained using the 397.007 nm line. In any case, the good agreement obtained without internal standardisation indicates a compound-independent ICP-OES response.

Table 2: Analytical figures of merit (with Tris solutions in DDW)

Analyte	Sensitivity (cps g of solution mol^{-1} analyte)	R^2	Detection limit (mol analyte g^{-1} solution)	Blank signal (cps, mean ± s.d., n= 5)
C	$(4.08 \pm 0.23) \times 10^9$	0.990	2×10^{-7}	23190 ± 260
N	$(6.69 \pm 0.27) \times 10^5$	0.997	5×10^{-5}	3616 ± 11
O	$(10.9 \pm 0.6) \times 10^4$	0.991	2×10^{-3}	1771 ± 69
H	$(4.89 \pm 0.32) \times 10^6$	0.992	1×10^{-3}	209400 ± 1900

Table 3: Analyte concentrations (± standard deviation, mol analyte g^{-1} glucosamine) in D-glucosamine hydrochloride (n=4)

Analyte, wavelength (nm)	Concentration		Student's	
	Measured	Expected	$t_{calculated}$	t_{table}
C, 247.856	0.0282 ± 0.0025	0.027825	0.3	3.182
H, 397.007	0.0702 ± 0.0077	0.064925	1.4	3.182
O, 130.485	0.0231 ± 0.0017	0.023188	0.1	3.182
N, 149.272	0.00787 ± 0.00046	0.0046375	14	3.182

The source of the discrepancy in the case of N is unknown and under investigation. Spectroscopic interference can be ruled out because similar results were obtained with different N emission lines (149.262 nm, 174.525 nm, 343.715 nm, 404.131 nm, 410.995 nm, 593.178 nm, etc.). Contamination of the torch, which might have resulted in an unduly high blank, was similarly ruled out because cleaning the torch injector in aqua regia made no significant difference to the blank signal. No significant difference was also observed when using bottles that had not been exposed to nitric acid.

Nonetheless, these results are very encouraging and demonstrate that ICP-OES may be used for the accurate determination of H, O and C in an organic compound dissolved in water if a simple weighed blank subtraction is carried out. Given that the only requirement in terms of sample preparation is dissolution of the compound in water, this ICP-OES method is clearly advantageous compared to HPLC-based methods with other detectors requiring derivatisation.[1,8,9] It also allows the simultaneous determination of trace elements, which is not possible by the latter methods. For example, several elements (Al, As, Fe, S, Cr, Na and Sb) could readily be detected in the solution of D-glucosamine hydrochloride.

Although 1 g of glucosamine was used in this work to obtain about 10 mL of solution so as to enable many tests, the amount of compound could be reduced if the final volume/mass of solution was also reduced. In addition, if the sample uptake rate was decreased, by switching to a micronebulizer for instance, the amount of solution required for analysis could be further reduced, thereby requiring even less organic compound.

Conclusions

This preliminary work reveals that ICP-OES with a conventional sample introduction system and a long torch can be used to accurately measure the concentrations of C, O and H in aqueous solutions, by a simple external calibration with Tris standard solutions without internal standardisation. These concentrations may be used to verify the identity of an organic compound if only one compound is present in solution and if the latter is prepared by weight so as to enable a suitable correction for the

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contribution of water to the O and H signals in particular. Hence, ICP-OES could be used to both confirm the identity of an organic compound in solution and assess its purity, as multi-element determination can be simultaneously performed.

Future work will further investigate the source of discrepancy in the case of N by looking at more nitrogen-containing compounds. Unless the problem is compound-dependent responses, accurate results should indeed be more readily obtainable for N than they are for H, given the much higher background in the latter case. Means to lower the detection limit and reduce sample consumption will also be explored in an attempt to make the approach competitive with conventional elemental analyzers. For example, flow injection will be used to significantly reduce sample consumption. This may then enable application of the method to newly synthesized organic compound.

In order for the method to be applicable to the analysis of nutraceuticals, which usually contain a binder in addition to the active compound, a separation will likely be required, which may be performed by HPLC with on-line detection by ICP-OES. While this separation is more time-consuming than straight nebulisation of a solution into the plasma, HPLC-ICP-OES would nonetheless be faster than previous methods requiring a derivatisation step,[1,8,9] while simultaneously providing information on trace elements present.

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