Analysis of Blood Serum on the Liberty Series II ICP OES with the Axially-Viewed Plasma

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

Andrew Ryan

Introduction

The role of major, minor and trace levels of elements in human health has been an important area of scientific research. In particular, research on the value of trace elements to the diagnosis, treatment and prevention of diseases has been vast.

The advent of atomic absorption (AA) techniques and the development of the graphite tube atomizer (GTA) has provided the means for accurate determination of all levels of many elements in human body fluids. An advantage of the graphite furnace is the small sample consumption in the determination of trace levels. Disadvantages of flame AA are that releasing agents or modifiers are necessary and careful control of the flame stoichiometry is important to overcome chemical interferences [1]. While the atomic absorption technique offers adequate performance, in most cases it is a single element technique and is therefore slow.

The inductively coupled plasma mass spectrometer (ICP-MS) offers rapid, highly sensitive, multi-element determinations. The high sensitivity of ICP-MS means that samples can be diluted to give a reasonable working volume. Dilution is also required for ICP-MS because of limitations imposed by the sample matrix. Typically with ICP-MS, an upper total dissolved solids (TDS) limit of 0.2% in the solution should not be exceeded to ensure continuous operation for an extended period [2]. At TDS levels in excess of this limit, unacceptable levels of signal instability are commonly experienced.

Inductively coupled plasma optical emission spectrometry (ICP-OES) also offers rapid, multi-element determinations. The sensitivity of ICP-OES is lower than that of either ICP-MS or AA-GTA, but ICP-OES can handle higher levels of TDS than ICP-MS and is much faster than AA-GTA. Since ICP-OES is able to analyze samples with higher TDS, more concentrated solutions can be prepared allowing trace elements to be measured. A disadvantage of ICP-OES for the determination of trace elements is that sample volumes will often be small and sample consumption for ICP-OES is

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typically about 1–2 mL/min. The use of a microconcentric nebulizer (MCN) is a convenient way to reduce the sample consumption. Such nebulizers are obtainable from several suppliers. For example, Glass Expansion Pty Ltd supply MCNs with free aspiration uptake rates ranging from 100 to 800 µL/min. A Glass Expansion MCN with a free aspiration uptake rate of 400 µL/min was used in this work.

This report describes the analysis of blood serum using standard quantitative calibration with aqueous standards. Viscosity effects of the blood serum solutions were corrected using scandium (361.384 nm—ionic line) as an internal standard. Major, minor and trace elements were determined in a single analysis.

A major element in blood serum is sodium, which is an easily ionized element (EIE) and has been reported to cause ionization interference when present in reasonably high levels. Ionization interference tends to cause a reduction in signal intensity with increasing concentration of EIE and the effect is prominent at interferent concentrations at or above 100 mg/L. The atomic lines of Na, K and to a lesser extent Ca (422.673 nm) exhibit signal enhancement with increasing concentrations of EIE. The effect can be easily minimized or eliminated on a radially-viewed ICP-OES by adjusting the viewing height. For the more sensitive axially-viewed ICP-OES, many reports of interferences due to EIE have been described [3–5]. Reducing the nebulizer pressure and increasing the RF power has been reported to reduce ionization interference on the axially-viewed ICP-OES [3]. Scandium as an internal standard has also been found to compensate for part of the signal depression [4,5]. Generally, when analyzing samples that contain high levels of EIE, it is recommended that all standards have similar levels of EIE added (matrix matching).

An alternative is to saturate the plasma with a high concentration of another EIE such as cesium. Therefore, the effect of adding cesium as an ionization buffer to the standards and samples was also investigated.

Cesium was chosen as an ionization buffer as it has a low energy of ionization, is not very sensitive by ICP-OES and, therefore, spectral interference is generally not a problem. Cesium chloride is available in a very pure form and does not build up in the torch injector tube as readily as other alkali salts.

The accuracy and validity of the method was assessed by the use of Nycomed Pharma "Seronorm Trace Elements Serum batch no. 311089".

Experimental

Instrumental

An Agilent Liberty Series II ICP-OES with the axially-viewed plasma was used for the analysis.

The Liberty Series II ICP features a 40 MHz free running RF generator, a 0.75 m Czerny-Turner monochromator with a 1800 grooves/mm holographic grating used in up to four orders. The resolution of the optical system ranges from 0.018 nm in the 1st order to 0.006 nm in the 4th order.

The instrument was controlled with a Digital Equipment Corporation (DEC) Venturis computer with an Intel Pentium processor and Agilent Plasma 96 software running under Microsoft Windows 95 operating system.

The instrument operating conditions are listed in Table 1.

Table 1. Instrument Operating Conditions

Power	1.0 kW
Plasma gas flow	15.0 L/min
Auxiliary gas flow	1.5 L/min
Spray chamber type	Glass cyclonic
Torch	Standard axial torch with 2.3 mm id injector
Nebulizer	High flow microconcentric nebulizer (Glass Expansion Pty Ltd), free aspiration uptake rate 400 µL/min
Nebulizer pressure	300 kPa
Pump tube	Inlet - PVC, orange-green, 0.38 mm id Cs solution inlet, orange-blue, 0.25 mm id Outlet - PVC, black-black, 0.76 mm id
Pump speed	15 rpm
Sample uptake rate	160 µL/min
Integration time	3 seconds for Ca, Cu, Fe, K, Mg, Na, P, S and Zn 5 seconds for Al and Mn
No. of replicates	3
Sample delay time	20 seconds
Stabilization time	15 seconds
Fast pump	On
Upward curvature limit	125%
Background correction	Polynomial plotted background for Ca, Cu, Fe, K, Mg, Na, P, S and Zn
	Offpeak background correction for Al and Mn
	0.015 nm left of peak
	0.015 nm right of peak
PMT voltage	650 V

For the determination of sulfur, an Auxiliary Gas Module-2 (AGM-2) is required. The AGM-2 provides a nitrogen purge for the monochromator to extend the working wavelength range from 189 nm down to 175 nm. The default grating order was used for all lines with the exception of the Al 396.152 nm line where the order was changed from 1st to 2nd order because of the presence of spectral interference from the blood serum matrix.

Standard Preparation

Aqueous standards were prepared from Custom-Grade Multi-element Solution Var Cal 2 (Inorganic Ventures, Inc.) and from 1,000 mg/L and 10,000 mg/L single element standards (Spectrosol, BDH Chemicals). The standards were made up in 18 MΩ Milli-Q water with 1% v/v high purity HNO_3 (Mallinckrodt, AR SELECT PLUS) and 0.01% v/v Triton X100 prepared from a 1% w/v Triton X100 solution. Scandium was added to each solution as an internal standard with a final concentration of 0.5 mg/L.

The following calibration standards were prepared.

Table 2. Calibration Standards

Standard No.	Concentration (mg/L)
Standard 1	20 µg/L Al, Cu, Fe, Mn and Zn 1.3046 mg/L P 6.6752 mg/L S
Standard 2	100 µg/L Al, Cu, Fe, Mn and Zn 6.5228 mg/L P 33.376 mg/L S
Standard 3	0.4 mg/L Ca and K 0.1 mg/L Mg 10 mg/L Na
Standard 4	2 mg/L Ca and K 0.5 mg/L Mg 50 mg/L Na
Standard 5	10 mg/L Ca and K 2.5 mg/L Mg 250 mg/L Na (for Na 330.237 nm line only)

Rinse and calibration blank solutions were prepared from 18 MΩ Milli-Q water with 1% HNO_3 and 0.01% Triton X100

Sample Preparation

Solutions were prepared from Seronorm Trace Elements Serum, batch no. 311089.

The serum was reconstituted by removing the screw cap and carefully lifting the rubber stopper—without removing it completely. Air was allowed to enter the vial through the grooves on the lower part of the stopper. The stopper was removed and 3.00 mL of 18 MΩ Milli-Q water was added. Care must be taken when removing the stopper to avoid loss of dried material. The vial was closed and allowed to stand for 30 minutes. The contents were completely dissolved by swirling gently. Shaking of the vial will result in the formation of foam. Long term contact between the liquid and the rubber stopper should be avoided, particularly for the determination of zinc or aluminium, to prevent contamination from the rubber stopper.

Three solutions with dilution factors of 5, 20 and 100 were prepared in 1% HNO_3 and 0.01% Triton X100.

Scandium was added to each solution as an internal standard with a final concentration of 0.5 mg/L.

For the study of the effect on the addition of cesium as an ionization buffer, 2% w/v Cs as CsCl was added online to all solutions by pumping the solution into a "T" piece just before the nebulizer. The optional three channel pump was utilized with one channel used to introduce the cesium solution. It is possible to add the internal standard to the cesium solution instead of each individual solution.

Results and Discussion

Wavelength Selection

Wavelength selection was based on the sensitivity of the line and the concentration of elements in each of the solutions. For most lines, spectral interference did not appear to be a major problem.

Ionic and atomic lines were selected for Ca and Mg so the effect of ionization interference on the two emission line types could be observed. Any variation in the results would also show the presence of spectral interference.

The K 766.490 nm line is known to be subject to spectral interference from Mg, so the K 769.896 nm line was also selected. The concentration of K in this sample was approximately 8.5 times that of Mg, and consequently the Mg spectral interference on the K 766.490 nm line was expected to be negligible. This expectation was confirmed when similar results were found for K at both lines.

For Cu, the 324.754 nm or 327.396 nm emission lines are generally used, although the 327.396 nm line is preferred. For both Cu lines, a small OH emission line from the aqueous matrix is observed and is more prominent with the axially-viewed plasma than with the radially-viewed plasma. The OH emission line is not resolved from the 324.754 nm line, which is used in the 2nd order (default or recommended setting), whereas the OH emission line is almost completely resolved from the 327.396 nm line, which is used in the 1st order.

Figures 1 and 2 show the wavelength scans for the Cu 327.396 nm and 324.754 nm emission lines in blood serum diluted by a factor of 20.

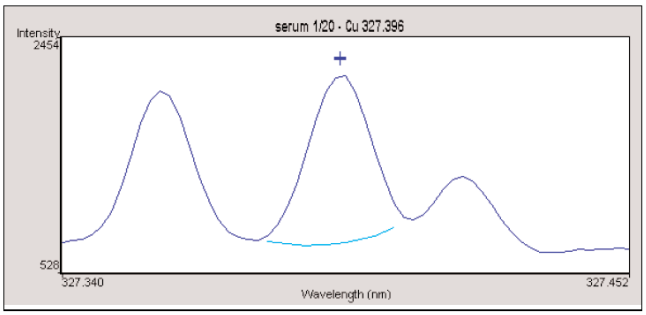

Figure 1. Wavelength scan for Cu 327.396 nm in the 1st order.

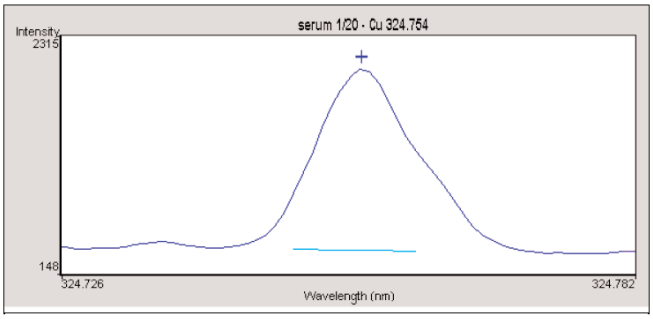

Figure 2. Wavelength scan for Cu 324.754 nm in the 2nd order.

The peak to the left of Cu (327.396 nm) is Sc and causes no problems as it is completely resolved. The tail of the OH peak does contribute slightly to the signal of the Cu (327.396 nm) but this has been successfully corrected using polynomial plotted background correction.

There is also the option of using the 327.396 nm line in the 2nd order where the resolution will be improved by a factor of 2. The sensitivity of the line is only reduced by approximately 30% when using it in second order and the peaks are completely resolved.

The Cu 327.396 nm line in the 1st order was selected for the analysis.

Blood Serum Analysis

The analysis consisted of a single Seronorm Trace Elements Serum, batch no. 311089, that was diluted 100-fold for the determination of Ca, Mg, Na and K, 20-fold for the determination of Ca, Mg, Na, K, Cu, Fe, P, S and Zn and 5-fold for the determination of Al and Mn. The elements Ca, Mg Na and K were determined in the 100 and 20-fold dilution blood serum solutions because any variation in the result would indicate the presence of ionization interference.

The analysis was repeated with the addition of Cs as an ionization buffer. The Cs was added by pumping CsCl solution (2% w/v Cs) into a "T" piece just before the nebulizer.

Blood serum contains high levels of sodium and the potential for ionization interference is high. Matrix matching, such as having equal amounts of Na in all solutions, would mean that any signal enhancement or suppression because of ionization interference would be the same for all solutions. To measure major, minor and trace levels of elements in blood would then require multiple analyses because blood serum solutions of various dilution factors would be necessary with matrix matched standards to be prepared for each. The aim of this work was to show that the effect of ionization interference could be overcome, and therefore allow all levels of elements to be determined in a single analysis.

Figures 3–10 represent the calibration graphs for the standard solutions displayed in Table 2, with and without the addition of cesium. Standards 3, 4 and 5 contained varying levels of Ca, K and Na. Sodium was present in concentrations high enough for ionization interference to have considerable influence on the signals of the other elements in the standard solutions.

Figures 3– 6 show the effect of the varying levels of ionization interference, because of the varying EIE concentration between solutions, on the atomic lines of K (766.490 nm and 769.896 nm), Na (589.592 nm and 330.237 nm) and Ca (422.673 nm) as signal enhancement has produced upward curvature of the calibration. The addition of Cs nullified the effect of the varying levels of ionization interference, producing a more linear calibration. Adding Cs instead of matrix matching allows all elements to be determined in a single analysis because sample solutions with varying dilution factors, and varying concentrations of EIE, can be analyzed.

An ionic line for Ca (317.933 nm) was also used and upward curvature of the calibration was not found. The calibration for Mg (285.213 nm) atomic line exhibited little, if any, upward curvature as did the remaining atomic and ionic analyte lines. This is consistent with other reports [4,5] that the atomic lines of group I and to a lesser extent, group II elements,

exhibit signal enhancement with increasing levels of EIE. The atomic lines of other elements and all ionic lines tend to exhibit signal suppression by EIE but the effect is not as severe.

In the Plasma 96 software, the maximum % error of the slope of the calibration, which is set in the calibration page of the method editor, only sets the limit of downward curvature for each specific element. The maximum % error of upward curvature is set from the switches registry (\\Varian\ICPAES\Run\Switches.reg) and is applied to all elements. The upward curvature limit was set to 125% and the calibration failed if the slope at the top of the curve was more than 125% of the slope at the bottom of the curve.

Without the addition of cesium, the upward curvature for the atomic lines of Na, K and Ca exceeded the limits and the calibration failed for these lines.

With the addition of cesium, the effect of ionization interference was reduced and all elements calibrated successfully.

Even though the calibrations for the atomic lines of Ca, K and Na failed when cesium was not added, the maximum upward curvature limit was increased post-run so that the calibrations would pass. By so doing, a comparison of the results with and without the addition of cesium could be made.

Note that Figures 3–10 show the effect of Cs on the linearity of the calibration and not the effect on the intensity of the analyte peak.

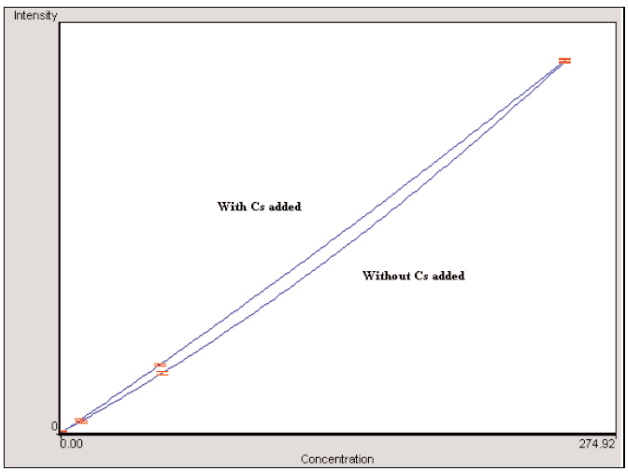

Figure 3. Calibration graph for Na 330.237 nm (atomic) with and without the addition of cesium.

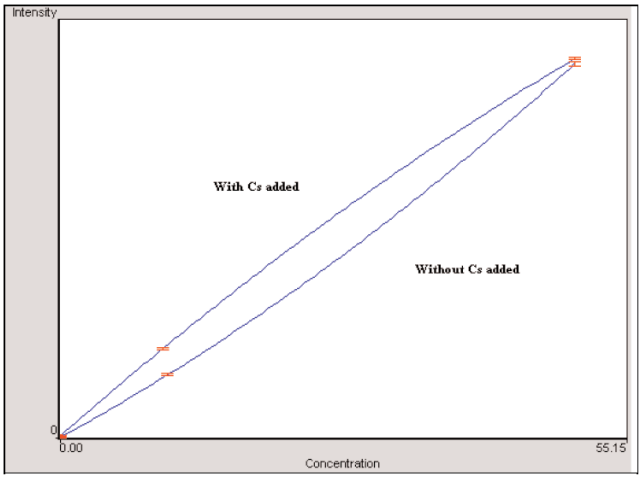

Figure 4. Calibration graph for Na 589.592 nm (atomic) with and without the addition of cesium.

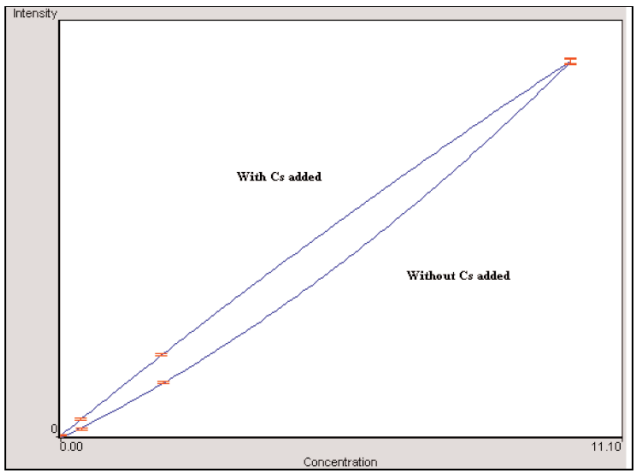

Figure 5. Calibration graph for K 766.490 nm (atomic) with and without the addition of cesium.

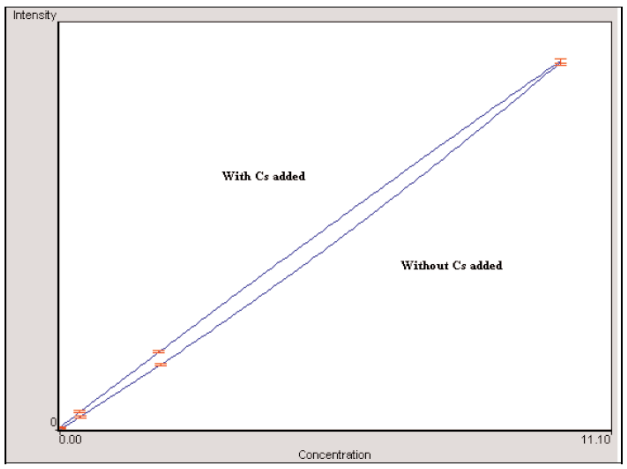

Figure 6. Calibration graph for Ca 422.673 nm (atomic) with and without the addition of cesium.

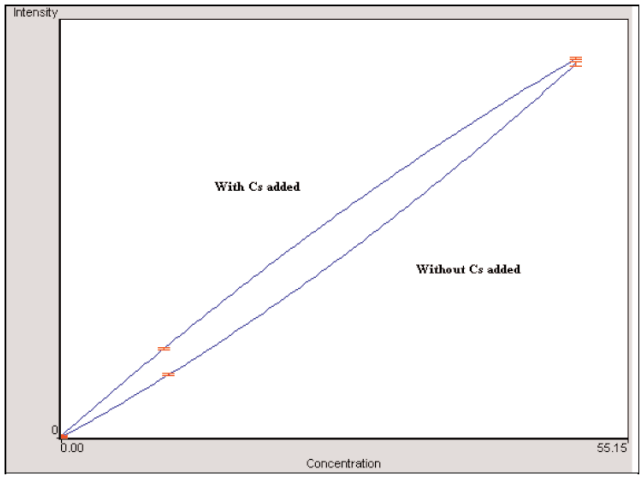

Figure 7. Calibration graph for Ca 317.933 nm (ionic) with and without the addition of cesium.

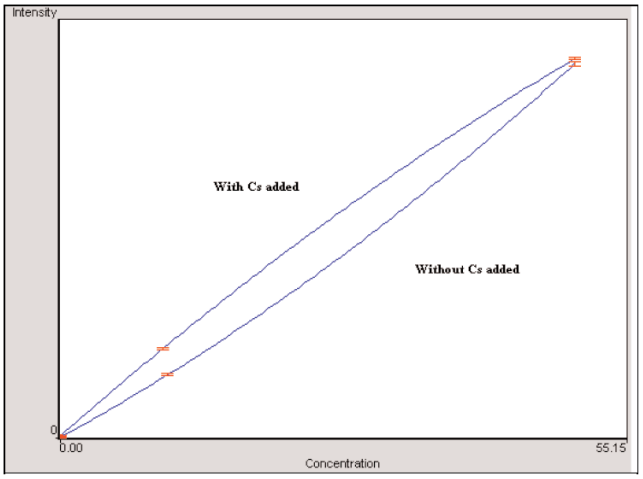

Figure 9. Calibration graph for Mg 285.213 nm (atomic) with and without the addition of cesium.

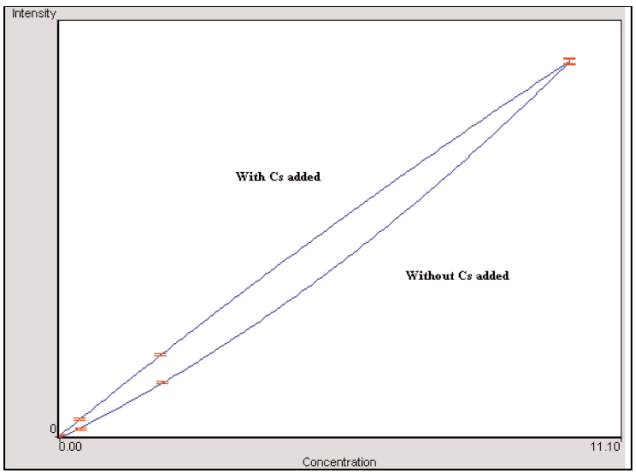

Figure 8. Calibration graph for Mg 279.553 nm (ionic) with and without the addition of cesium.

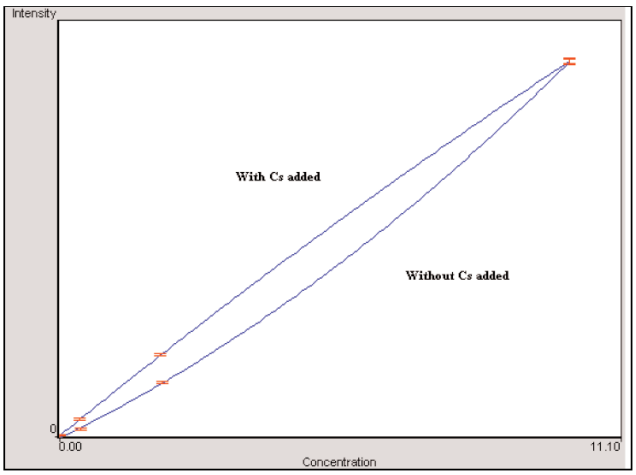

Figure 10. Calibration graph for Cu 327.396 nm (atomic) with and without the addition of cesium.

The mean results of the triplicate analyses for the determination of elements in blood serum with and without the addition of 2% w/v Cs are listed in Table 3.

Table 3. Results of the Blood Serum Analysis With and Without the Addition of Cesium

Element	Wavelength (nm)	Blood serum dilution factor	Measured value without Cs (mg/L)	Measured value with Cs (mg/L)	Certified value (mg/L)
Ca	317.933	100	94.9 ± 2.0	95.1 ± 0.8	94
Ca	317.933	20	96.4 ± 2.0	95.4 ± 1.7	94
Ca	422.673	100	94.1 ± 0.9	93.9 ± 0.7	94
Ca	422.673	20	103.2 ± 0.6	95.7 ± 1.5	94
Mg	279.553	100	19.0 ± 0.1	20.0 ± 0.2	20
Mg	279.553	20	19.3 ± 0.3	20.0 ± 0.1	20
Mg	285.213	100	19.3 ± 0.3	19.7 ± 0.2	20
Mg	285.213	20	19.9 ± 0.3	19.6 ± 0.1	20
Na	330.237	100	3101 ± 7	3151 ± 45	3080
Na	330.237	20	3314 ± 60	3307 ± 6	3080
Na	589.592	100	3305 ± 49	3166 ± 40	3080
Cu	327.396	20	1.19 ± 0.01	1.24 ± 0.01	1.27
Fe	259.940	20	1.19 ± 0.02	1.28 ± 0.04	1.3
K	766.940	100	151.2 ± 3.0	163.8 ± 2.6	168
K	766.940	20	162.8 ± 0.02	167.0 ± 0.8	168
K	769.896	100	154.5 ± 4.0	168.6 ± 3.1	168
K	769.896	20	164.3 ± 3.4	170.7 ± 0.7	168
P	213.618	20	75.5 ± 0.8	75.5 ± 1.1	–
S	180.731	20	1077 ± 6	1112 ± 17	–
Zn	213.856	20	1.51 ± 0.03	1.58 ± 0.03	1.50
Al	396.152	5	0.090 ± 0.005	0.088 ± 0.012	0.093
Mn	257.610	5	0.0073 ± 0.0003	0.0077 ± 0.0004	0.0073

The effect of ionization interference, particularly on the EIE such as K, Na and Ca, is clearly visible from the results displayed in Table 3. Without the addition of Cs, variations in the measured results were found for the atomic lines of Ca (317.933 nm), Na (330.237 nm) and K (766.940 nm and 769.896 nm). The results for the 100-fold dilution blood serum solution were lower than those measured in the 20-fold dilution blood serum solution because of the higher level of EIE in the latter, particularly Na.

The effect of ionization interference on different line types was observed for the Ca atomic line (422.673 nm) and Ca ionic line (317.933 nm). From Figures 6 and 7, it can be seen that ionization interference had considerable effect on the calibration of the Ca (422.673 nm) line while the Ca (317.933 nm) line remained unaffected. This is reflected in the results of Table 3 with varying results found for Ca (422.673 nm) and similar results found for Ca (317.933 nm) at the different dilution factors, without the addition of Cs. When Cs was added, the measured values were similar for both Ca lines and both diluted solutions.

In comparison, the effect of ionization interference on the Mg (279.553 nm) ionic line and Mg (285.213 nm) atomic line was small, but still present. Similar results were obtained for both the ionic and atomic lines of Mg at the different dilution factors, although slight enhancement of the Mg (285.213 nm) line in the more concentrated solution was observed. The addition of cesium appeared to improve accuracy of the result for the Mg (279.553 nm) ionic line, suggesting a small amount of signal suppression due to ionization interference.

Some signal depression was also observed for Cu and Fe, although it was not severe. With the addition of cesium, the determined concentrations were closer to the certified values.

With the addition of cesium, Na still appears to be affected by ionization interference when the levels of EIE are high. This is observed for the Na (330.237 nm) as a higher result was found for the more concentrated blood serum solution. It would therefore be recommended that blood serum be diluted by a least a factor of 100 when repeating the analysis to determine Na.

The determination of sodium was repeated with a 1000-fold dilution of the blood serum. Standards containing 1 and 5 mg/L Na only were prepared and the Na 589.592 nm line was used. No internal standard or ionization buffer was added. Triplicate analyses were done and the average result was 3181 mg/L in the original sample. The analysis was repeated using a standard high flow concentric nebulizer and the average result was 3170 mg/L. Both these results are similar to that obtained for Na (330.237 nm and 589.592 nm) in the 100-fold dilution blood serum solution with cesium being added.

The measured concentrations of Zn and the trace elements Mn and Al without the addition of cesium were similar to the certified values. With the addition of cesium, a slight increase in the measured concentrations of Zn and Mn were found. Although the results for Zn and Mn were slightly higher, they still compare well with the certified values.

No certified value was available for P and S in the blood serum batch that was used for the analysis. The measured results for P and S were however, very close to the certified values of another Seronorm Trace Elements Serum batch. The certified concentrations of the other elements for both serum batches varied only slightly and, therefore, the same could be assumed for P and S. These two elements did not appear to be affected by the presence of EIE as similar results were found with and without the addition of Cs.

Long Term Stability

No extensive evaluation of the long term stability was done with the microconcentric nebulizer. It appeared to operate well with no blockage being evident. Blood serum solutions diluted by a factor of 5 were aspirated continuously for periods of more than 30 minutes with no blockage being observed. Blood serum diluted 2-fold appeared to cause no problems.

A single long term stability run was done by continuously aspirating a 20-fold dilution blood serum solution and measuring the signal for a number of elements at intervals. The reproducibility of the measurements for Ca, Cu, Fe, Mg, Na, S and Zn over one hour ranged between 0.6 and 1.0 %RSD.

The replicate precision using a 3 second integration time and measuring 3 replicates ranged between 0.1 and 1.7 %RSD for all elements.

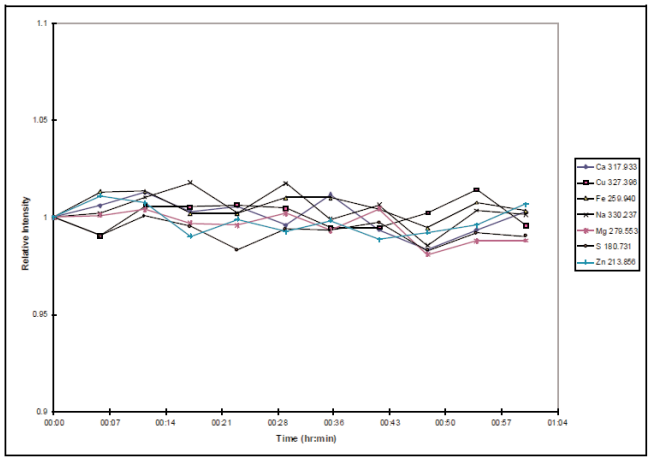

Figure 11. Signal stability over one hour for a 20-fold dilution blood serum solution.

Summary

The concentrations of major, minor and trace levels of elements in blood serum were determined in a single analysis on the Liberty Series II with the axially-viewed plasma.

Aqueous calibration solutions were used and the scandium internal standard successfully corrected for the varying viscosity of the sample. Scandium also exhibits signal suppression because of ionization interference and therefore compensates for part of the signal suppression of the other elements.

The addition of cesium as an ionization buffer considerably reduced the effect of ionization interference and the need for dilution, allowing both major, minor and trace constituents to be measured in a single analysis.

With the addition of cesium, all measured values were in very good agreement with the certified values for the Seronorm Trace Elements Serum sample, confirming the accuracy of the method.

The microconcentric nebulizer performed very well with no blockage ever occurring during the analysis of the blood serum. Sensitivity of the microconcentric nebulizer with an uptake rate of 160 µL/min was estimated as approximately half that of the standard high flow concentric nebulizer operating at an uptake rate of 1.5 mL/min. The sensitivity could have been improved by increasing the uptake rate but 160 µL/min appeared to be a good comprise between sufficient sensitivity, particularly for Al, and low sample consumption.

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