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Analysis of Blood Serum for Essential Metals by Simultaneous Multielement Atomic Absorption Spectrometry with Flame Atomization

Susan A. Lewis and Thomas C. O'Haver*

University of Maryland, College Park, Maryland 20742

James M. Harnly

Nutrient Composition Laboratory, U.S. Department of Agriculture, Beltsville, Maryland 20705

Blood serum is analyzed for Na, K, Ca, Mg, Fe, Cu, and Zn by using a simultaneous, multielement, atomic absorption spectrometer with a continuum source (SIMAAC). Because of the extended range capabilities of the SIMAAC system, analyses can be carried out on a single 5-fold dilution. Standards and samples are matched in appropriate concentrations for the major, minor, and trace elements. The SIMAAC results show excellent agreement with the certified values for the National Bureau of Standards serum reference material and the manufacturer's values of a commercially available calibration material, Cation-Cal (American Dade). The relative standard deviations of day-to-day precision studies range from 1.4% for Na to 11.1% for Fe. Good correlations are obtained between the values obtained by SIMAAC and comparative clinical methods.

The analysis of serum for essential metals is of major importance in the clinical laboratory. Evenson (1) has stated that the trend in clinical chemistry, with regard to essential metals, has moved from disease states and toxicology into the area of nutrition, reference values, and health maintenance. Mertz (2) has emphasized the necessity for methods that are specific, precise, and sensitive for essential metals. In his review, Faulkner (3) noted the importance of analytical techniques in the determination of nutritionally essential trace metals. Multicomponent analyses are common in the clinical laboratory and are preferred for their efficient use of time and sample and their cost effectiveness. However, many of the essential metals are determined by single-element techniques. Multielement techniques such as neutron activation analysis and X-ray fluorescence are impractical in a routine clinical laboratory for reasons of time, cost, specimen size, preparation, and handling. The three optical techniques upon which multielement techniques can be based—emission, fluorescence, and absorption—have been compared in several reviews (4-7). Emission is generally considered the most fundamentally suited for multielement analysis; with the introduction of inductively coupled plasmas, emission techniques are entering a renaissance (8). Atomic fluorescence is not widely used for routine analysis; however with the advent of the first commercially available instrument (Baird Corp., Bedford, MA), this technique will probably be more fully utilized. However, atomic absorption spectrometry (AAS) is by far the most widely used and well accepted of these techniques in the clinical laboratory, but it is not generally considered a multielement technique. We describe the use of a prototype simultaneous multielement atomic absorption spectrometer (SIMAAC) to determine the seven most often determined metals in serum. The average concentrations of these metals in serum range from 1 to 3500 mg/L. The use of conventional AAS would not permit the determination of these metals on

a single dilution, due to this wide concentration range. The SIMAAC system can cover 4 to 6 orders of magnitude of concentration by the use of multiple analytical curves (9). We report the simultaneous analysis of serum for Na, K, Ca, Mg, Fe, Cu, and Zn by using the SIMAAC system with air-acetylene flame atomization.

EXPERIMENTAL SECTION

Instrumentation. The SIMAAC system has been described in detail elsewhere (10). It consists of a continuum source, an atomizer (either flame or furnace), a 20-channel Echelle polychromator, which produces a two-dimensional spectral array, a quartz refractor plate for wavelength modulation, and a dedicated minicomputer. Wavelength modulation provides double-beam performance and background correction at all wavelengths.

Reagents. Two stock, custom-made, mixed standards (Spex Industries, Metuchen, NJ) containing 6000 mg/L Na, 300 mg/L K, 200 mg/L Ca, and 100 mg/L Mg and 100 mg/L Fe, Cu, and Zn, respectively, were combined to give five standards that bracket the physiological ranges of the metals under study in blood serum. The five standards have base concentrations of: Na (3000 mg/L), K (150 mg/L), Ca (100 mg/L), Mg (50 mg/L), Fe, Cu, and Zn (2 mg/L), with dilution factors of 0.1, 0.4, 0.8, 1.2, and 1.6. These standards were then diluted 5-fold with a diluent containing 50 ml/L glycerol (11) (Sigma Chemical Co., St. Louis, MO) and 1 g/L lanthanum (12) as chloride (Fisher Scientific Co., Silver Spring, MD) in 18 M Ω deionized water (Millipore Corp., Bedford, MA). Viscosity differences between the standards and samples are minimized by use of a glycerol diluent for the standards. The potential chemical interference of phosphate for calcium and magnesium determinations is reduced by the addition of lanthanum to the sample and standard diluents. The National Bureau of Standards human serum SRM 909 and a commercially available, nonhuman, protein-based calibration reference material, Cation-Cal (American Dade, Miami, FL) were used as quality control materials.

Procedure. The NBS SRM 909, Cation-Cal, and serum samples of 0.5 mL were diluted 5-fold with 1 g/L aqueous lanthanum. Samples and diluted standards were analyzed in triplicate with blanks of 1 g/L La and 1 g/L La plus 50 mL/L glycerol, respectively. By use of flame atomization and the SIMAAC system, samples can be analyzed at a rate of 80-90 per hour. Optimum results were obtained at observation heights 1.5 mm above the burner head with an air-acetylene flame (air = 12.5 L/min, acetylene = 2.6 L/min) (13).

Comparative Methods. The methods used for comparison to SIMAAC were as follows. In the first part of the study we used methods that were currently in use in a local clinical laboratory. Na and K were determined with ion-selective electrodes (ISE) by the Ektachem analyzer (Kodak, Rochester, NY). Colorimetric methods were employed for the determination of Ca with Arsenazo 3 on the Ektachem analyzer, Mg with Calmagite (Pierce Chemical Co., Rockford, IL) on a Cobas centrifugal analyzer (Hoffman La Roche, Nutley, NJ), and Fe with Ferrozine (Harleco, Gibbstown, NJ) on a spectrophotometer (Perkin-Elmer, Norwalk, CT). Zn was determined by air-acetylene AAS against glycerol-based standards (11) using a Perkin-Elmer 303 and Cu by furnace AAS (14) using a Perkin-Elmer 603.

Table I. Results of Analysis of Quality Control Materials by SIMAAC ($n = 10$)

	SRM 909				Cation-Cal			
	% RSD			certified value ^a	% RSD			manufacturer's value
	mean	within-run	day-to-day		mean	within-run	day-to-day	
Na, mmol/L	133	1.5	1.4	134.1 (+4.6) (-2.8)	153	1.4	1.4	156 ^b
K, mmol/L	3.47	1.8	2.9	3.52 (+0.19) (-0.06)	5.66	1.6	3.7	6.00 ^b
Ca, mmol/L	3.00	1.0	1.8	3.02 (+0.17) (-0.06)	2.91	1.7	1.7	2.85
Mg, mmol/L	1.23	0.9	4.0	1.21 (± 0.07)	2.83	1.7	2.1	2.90
Fe, mg/L	1.74	9.8	11.1	c	1.94	9.0	11.0	2.02
Cu, mg/L	1.04	2.8	5.7	c	2.05	2.5	4.8	2.17
Zn, mg/L	1.28	7.8	8.0	c	2.75	7.2	8.4	2.78

^a Value given as mean (95% confidence limits). ^b Value given for emission only. ^c Not certified.

Table II. Regression Statistics Comparing Results Obtained by the SIMAAC System with Those by Routine Clinical Methods

analyte	clinical method	n	Deming regression line	r	SE
Na, mmol/L	Ektachem (ISE)	20	$y = -1.71 + 1.02x$	0.899	1.59
K, mmol/L	Ektachem (ISE)	20	$y = 0.22 + 0.92x$	0.949	0.18
Ca, mmol/L	Ektachem (Arsenazo-3)	20	$y = 0.12 + 0.93x$	0.962	0.06
Mg, mmol/L	Cobas (Calmagite)	20	$y = -0.16 + 1.38x$	0.978	0.09
Fe, mg/L	colorimetric (Ferrozine)	20	$y = -0.11 + 1.31x$	0.857	0.31
Cu, mg/L	furnace AAS	20	$y = -0.11 + 0.98x$	0.959	0.12
Zn, mg/L	flame AAS (glycerol based stds)	20	$y = -0.09 + 0.92x$	0.966	0.11

Table III. Regression Statistics Comparing Results Obtained by the SIMAAC System with "Reference" Methods

analyte	reference method	n	Deming regression line	r	SE
Na, mmol/L	flame photometry after 200-fold dilution	44	$y = -0.19 + 0.99x$	0.989	1.75
K, mmol/L	flame photometry after 200-fold dilution	44	$y = -0.08 + 1.00x$	0.995	0.10
Ca, mmol/L	flame AAS after 50-fold dilution	44	$y = 0.04 + 0.97x$	0.975	0.06
Mg, mmol/L	flame AAS after 50-fold dilution	44	$y = -0.02 + 0.98x$	0.998	0.02
Fe, mg/L	flame AAS after deproteinization	40	$y = -0.04 + 0.99x$	0.987	0.10
Cu, mg/L	flame AAS after deproteinization	46	$y = -0.01 + 1.02x$	0.992	0.05
Zn, mg/L	flame AAS after deproteinization	56	$y = -0.09 + 1.10x$	0.975	0.10

In the second part of the study, other well-accepted or reference methods were used. Na and K were determined by flame photometry (Beckman Instruments, Carlsbad, CA), Ca and Mg by flame AAS, after a 50-fold dilution (12), and Fe, Cu, and Zn by flame AAS after deproteinization (12, 15), using a Perkin-Elmer 603.

RESULTS AND DISCUSSION

The results obtained for the determination of Na, K, Ca, Mg, Fe, Cu, and Zn in NBS SRM 909 and Cation-Cal, using the SIMAAC system, are shown in Table I, expressed as means, and within-run and day-to-day relative standard deviations (RSD). The means of the NBS SRM 909 obtained by SIMAAC are all within the 95% confidence levels set by NBS. The linear regression equations, correlation coefficients (r), and standard errors (SE) of the mean values obtained by SIMAAC vs. the certified and manufacturer's values for the NBS SRM 909 and Cation-Cal are $y = -0.08 + 0.99x$ ($r = 0.999$, $SE = 0.53$) and $y = 0.37 + 0.98x$ ($r = 0.999$, $SE = 2.05$), respectively.

The relative standard deviations are low (<3% for within-run and <6% for day-to-day) for all the elements except Zn and Fe. This lack of precision for these metals with the SIMAAC is due to the poor signal-to-noise ratio for Zn and lack of sensitivity for Fe. The precision of the data obtained using SIMAAC will be discussed later in this text.

In the first part of the comparison of methods study, 20 serum samples were submitted as unknowns to a local clinical laboratory and analyzed by their methods currently in use. The correlation plots comparing the results for the seven metals obtained by the SIMAAC and these methods are shown in Figure 1. Regression analyses are carried out by using the

Table IV. Comparison of Performance Limits (95% Confidence Level)

analyte	ref range	max acceptable performance limits ^a	performance limits ^b	
			SIMAAAC method	ref
Na, mmol/L	135-145	2.7	3.5	3.5
K, mmol/L	3.5-5.0	0.38	0.27	0.20
Ca, mmol/L	2.24-2.74	0.12	0.07	0.07
Mg, mmol/L	0.76-1.08	0.08	0.07	0.07
Fe, mg/L	0.50-1.50	0.25	0.23	0.14
Cu, mg/L	0.70-1.50	0.20	0.11	0.11
Zn, mg/L	0.68-1.21	0.13	0.11	0.13

^a References 18-20 (one-fourth of reference range).

^b Standard deviation of a mid-reference-range control $\times 1.96$.

Deming method (16, 17), which takes into account the error in the ordinate or reference values as well as the error in the experimental values, as opposed to conventional regression analyses, which assume no error in the reference values.

The regression statistics are shown in Table II. Ideally, slopes should have a value of one, the intercepts zero, the correlation coefficients one, and standard errors should be small compared to the range of values for the analyte. The colorimetric methods for iron and magnesium show large proportional errors as compared to SIMAAC, reflected by the slopes of 1.31 and 1.38, respectively. The colorimetric method for magnesium has been documented to be nonlinear above 2 mmol/L. The colorimetric iron method is linear up to 5 mg/L but has been shown to have interferences. It should

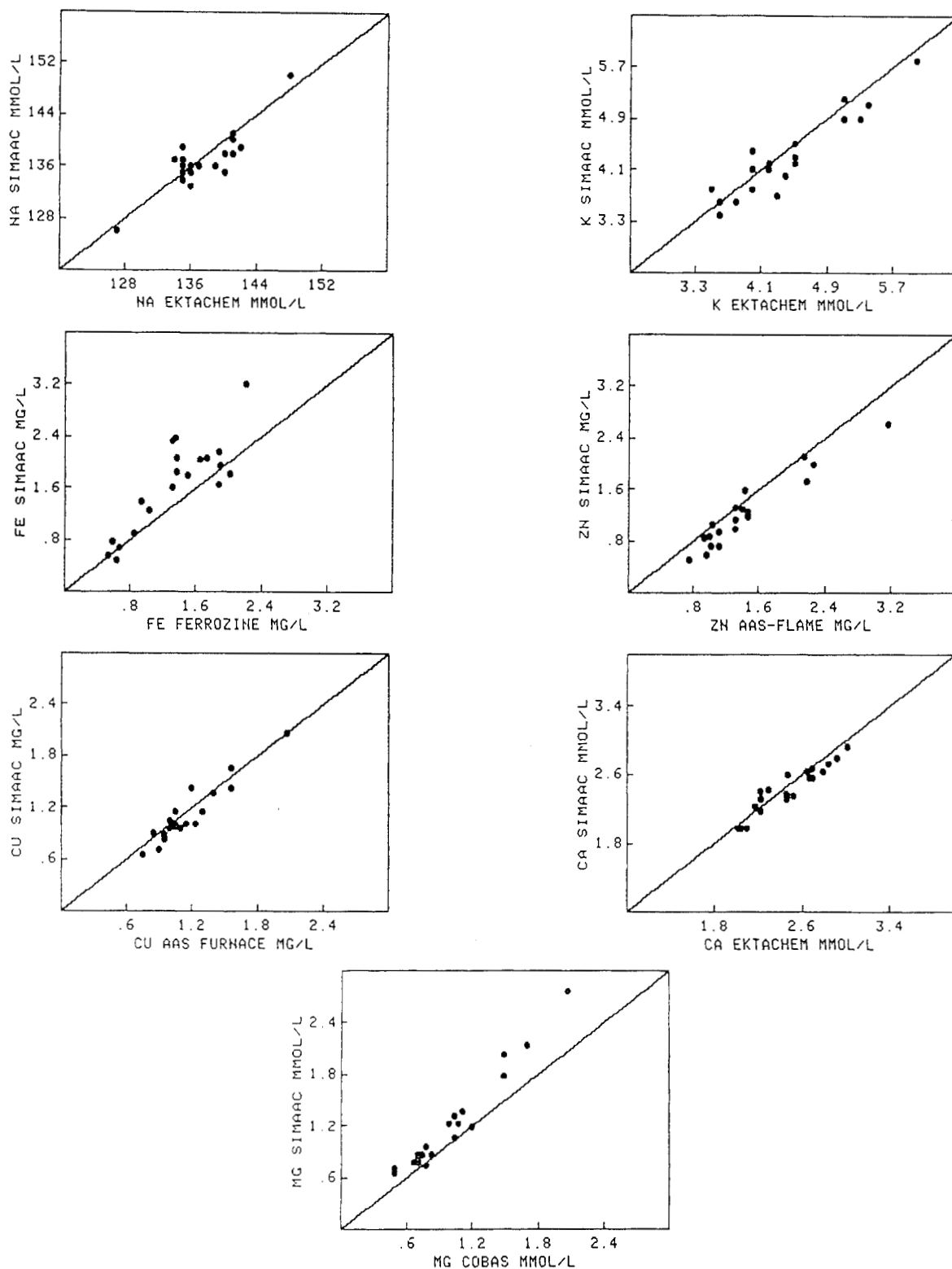


Figure 1. Correlation plots comparing results obtained by the SIMAAC system with those by routine clinical methods.

be noted that the correlation coefficient is adversely affected by a limited dispersion of points as is the case with sodium. We felt that a satisfactory conclusion could not be drawn concerning the performance of SIMAAC from these comparisons.

Westgard et al. (18) stated that the methods to which the experimental method, in this case SIMAAC, is compared must be carefully selected, as the interpretation of the experimental results depends on the quality of the comparative method. We therefore selected atomic spectrometric methods for the second part of the comparison of methods studies. Additional serum samples were analyzed by either flame photometry (Na

and K) or flame AAS and by the SIMAAC system. Correlation plots for the determination of the seven metals under study by SIMAAC and these methods are shown in Figure 2. The regression statistics are shown in Table III. It can be seen that the slopes vary from the ideal by less than 3% for all of the metals except Zn which is 10%. The intercepts and standard errors are small in comparison to the range of values for the analytes, and the correlation coefficients range from 0.998 for Mg to 0.975 for Zn and Ca.

In order to assess whether or not the precisions of the values obtained by SIMAAC are satisfactory for clinical determinations, we calculated 'performance limits' for SIMAAC and

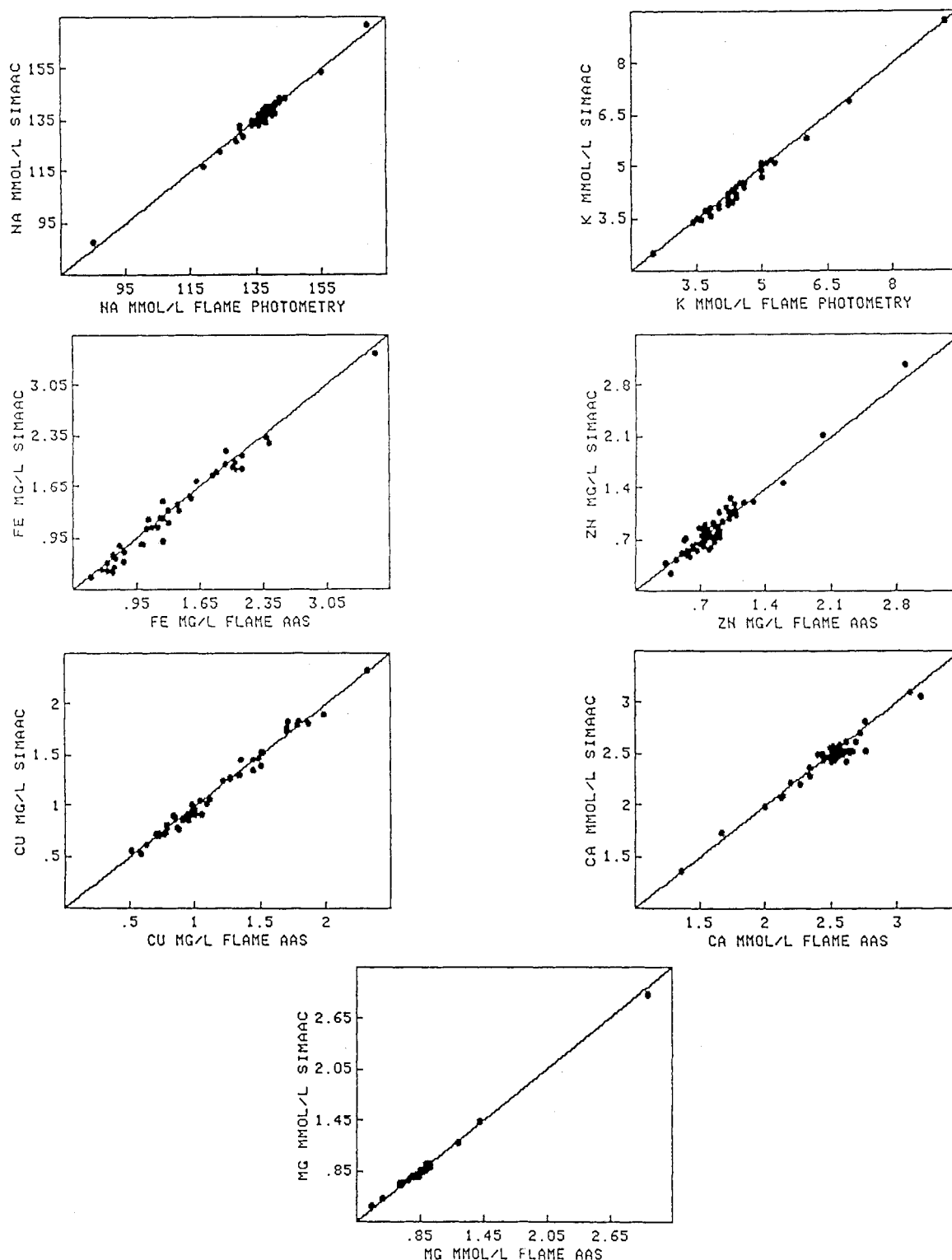


Figure 2. Correlation plots comparing results obtained by the SIMAAC system with those by "reference" methods.

the comparative methods shown in Table III, based on Westgard's review (18) of the work by Tonks (19, 20). Performance limits at the 95% confidence level are calculated by multiplying the standard deviations of a mid-reference-range control by 1.96. These limits are then compared to maximum 'performance limits' as defined by Tonks as being one-fourth of the reference range (i.e., range of normal values in human serum). Ideally the performance limits for the SIMAAC and the comparative methods should be less than these maximum limits. It can be seen from Table IV that all methods are clinically acceptable including SIMAAC for Fe and Zn, except *both* methods for Na. Westgard (18) states that in such cases, these methods may reflect the "state-of-the-art" limits and it is better to judge methods comparatively

rather than to judge all methods as unacceptable. In fact, the 95% confidence level for the Na value given by NBS for its reference material SRM 909 also does not meet the acceptable performance limits defined by this method (7.5 mmol/L as opposed to a maximum acceptable value of 2.7 mmol/L).

The ultimate criterion by which any analytical technique is judged is its ability to analyze real-life samples. Results obtained by using SIMAAC compare favorably to comparative methods, with the added advantage of simultaneous determinations for seven metals in a single sample.

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Registry No. Na, 7440-23-5; K, 7440-09-7; Ca, 7440-70-2; Mg, 7439-95-4; Fe, 7439-89-6; Cu, 7440-50-8; Zn, 7440-66-6.

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Atomization Mechanisms for Barium in Furnace Atomic Absorption Spectrometry

D. L. Styris

Pacific Northwest Laboratory, P.O. Box 999, Richland, Washington 99352

Atomic absorption spectrometry and mass spectrometry are used simultaneously in order to elucidate atomization mechanisms of barium dichloride in pyrolytic graphite, vitreous carbon, and tantalum furnaces. Gas-phase barium dicarbide is observed to appear concurrently with the free barium. Barium oxide and barium dihydroxide precursors appear with the chlorides. Surface reactions involving species that are adsorbed on the various furnaces are postulated to explain the appearances of the species that are observed in the gas phase.

It is well established that furnace materials can dramatically influence the sensitivities and detection limits observed in furnace atomic absorption (AA) spectrometry results. The large difference observed in sensitivities for barium vaporized from tantalum and from pyrolytic graphite furnaces exemplifies this effect. In this case, Renshaw (1) observed a 20-fold increase in sensitivity for Ba, in a solution of HClO₄ when pyrolytic graphite was replaced by tantalum as a furnace material. The detection limit that Renshaw measured for the tantalum furnace was 100 pg. Suzuki and Ohta have recently monitored strong barium AA spectra for 100 pg loadings of Ba in molybdenum furnaces (2). Hwang, Mokeler, and Ullucci report 10-pg detection limits for Ba vaporized from tantalum ribbon (3). L'vov obtained a 100-pg sensitivity for Ba in tantalum-lined graphite tube furnaces (4).

L'vov, Katskov, Kruglikova and Polzik suggest that the poor sensitivity for barium for the graphite furnace is a result of the formation of low volatility carbides that are intercalated in the graphite (5). This is based on their semiempirical estimates of heats of formation of carbides for 28 elements. Details of these estimates appear in L'vov's review of electrothermal atomization (6). Sensitivity is low because barium that is trapped as a carbide between basal planes of pyrolytic

graphite is no longer available for atomization (7, 8). Calculations of heats of formation suggest that all of the group 2A elements are likely to form these carbides (6). How this formation is achieved from the salt is not clear. Nickel (9) presents thermodynamic arguments for formation of the carbide from the metal oxide in a graphite furnace. Sturgeon, Chakrabarti, and Langford (10) hypothesize that metal chlorides and oxides are thermally dissociated in the AA furnaces. This can occur and carbide formation might follow if a portion of the resulting free barium were to react with the surface of the graphite furnace. In this case, Salmon and Holcombe (11) remind us that active surface site availability depends on concentrations of reactive species, such as oxygen, that are competing with the analyte for surface sites.

Other effects, such as ionization within the furnace may account for low sensitivities. Ionization of barium in graphite furnaces has been investigated by Sturgeon and Berman (12). These investigators conclude, from ion absorption and emission measurements, and from microwave attenuation results, that the degree of ionization of barium is low for furnace temperatures up to 2550 K. Hence, ionization will be a problem to barium AA only if atomization occurs at temperatures greater than 2550 K.

Observations based on AA spectra and thermodynamic data provide valuable insight to mechanisms leading to atomization (9, 11, 13-15). But intermediate species involved are not observed and can only be surmized in this case; at high temperatures the possible intermediates are numerous (16). Intermediates have recently been observed directly by using mass spectrometry (MS) techniques simultaneously with AA spectrometry. Styris and Kaye used this method to investigate rubidium atomization from tantalum furnaces (17) and vanadium atomization from vitreous carbon and from tantalum furnaces (18). More recently, Sturgeon, Mitchell, and Berman (19) used a similar approach to measure vapor pressures associated with intermediates that appear in conjunction with