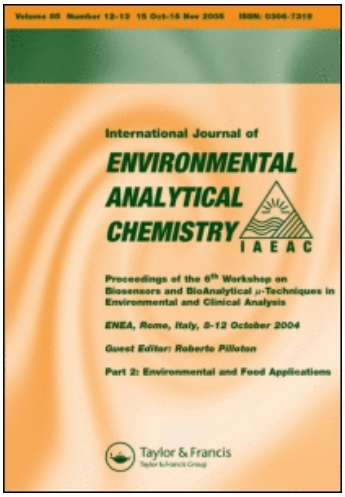

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Perspectives of Analysis of Carcinogenic and Mutagenic Metals in Biological Samples

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Perspectives of Analysis of Carcinogenic and Mutagenic Metals in Biological Samples†

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There is a continuing need to assess the status of exposure to humans of the carcinogenic and/or mutagenic metals in both biological and environmental samples to better ensure that current or past exposures do not entail unacceptable health risks or to detect potentially excessive exposure before the appearance of adverse health effects.

In order to more readily evaluate both the extent of exposure and trends of human exposure as well as the bioavailability, bioaccumulation and transport of these elements and their compounds, sensitive analytical procedures are required for their determination of the various oxidative states (as well as their organic derivatives) in complex matrices such as those found in both environmental and biological samples.

The major objective of this overview is to highlight the more recent trends and state-of-the-art methodologies for the determination and speciation of arsenic, selenium, cadmium, chromium and nickel in human and animal tissues while concomitantly noting germane aspects of their bioavailability and interactions.

Aspects of biological monitoring of the above elements will be stressed due to their potential utility in augmenting diverse epidemiologic and occupational health studies.

KEY WORDS: Arsenic, cadmium, chromium, nickel, selenium, environmental samples, body fluids, determination, speciation, bioavailability.

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INTRODUCTION

There is an acknowledged continuing need to assess the status of exposure, both nationally and globally, to humans of the carcinogenic and/or mutagenic metals in a spectrum of environmental and biological samples to better ensure that current or past exposures do not entail unacceptable health risks or to detect potentially excessive exposure before the appearance of adverse health risks. Although the elemental composition of body fluids and tissues is indicative of both the nutritional and pathological status of man, it is generally acknowledged that with few exceptions these data are lacking for many of the major, minor and trace elements. Much of the data available is of limited use and, for many tissues, virtually no data are available.

The major objective of this overview is to highlight the more recent trends and state-of-the-art methodologies for the individual, as well as multi-element determination and speciation of arsenic, selenium, cadmium, chromium, and nickel in human and animal tissue while concomitantly noting germane aspects of their bioavailability and interactions. Additionally, aspects of biological monitoring of the above elements will be stressed due to their potential utility in augmenting diverse epidemiologic and occupational health studies. Initially, it is important to note a number of considerations that have a direct bearing on tissue element concentrations.

ANALYTICAL CONSIDERATIONS

A number of recent reviews have stressed the principal considerations attendant with trace metal analysis that are particularly germane to the determination of toxic metals as well as essential metals in biological tissues.[107,122,132,182,245,261,294]

The nature of the tissue sample and its pre-treatment for subsequent measurement of the elemental content is the initial analytical consideration to be made noting that in general the three basic tissues are: body fluids, soft tissues and hard tissues with each of these matrices having different problems of sampling and collection, storage and sample pretreatment.[107,122,144,256,261,294]

There is broad agreement that analytical data for the levels of

trace elements in tissues and body fluids of humans cannot be accepted *a priori* as accurate unless *all* details of the analytical procedure are *critically* evaluated, including those of sample collection, preparation and digestion. Although numerous methods and procedures have been used to measure trace elements in the more readily available biological materials and the analytical quality of measurements may be excellent, little if any attention was given to sample collection, preparation, and making certain that samples are not contaminated during *all* procedures to which they were subjected. In many cases the reported values for the same element in the same substances vary over a wide range. This is often indicative of sample contamination, although in many cases it is also due to inaccuracy of the analytical methods. Additionally it should be noted that many reported methods and procedures do not include adequate verification of their accuracy.[107,122,182,256,295]

The fact that widely divergent reference values have been reported for trace elements in blood plasma or serum of *healthy* individuals can best be illustrated in Table I which catalogues chromium levels in human blood plasma or serum as reported in 42 studies from 1956 to 1983.[295] Impressive evidence has been collected to show that most of the discrepancies could be due to inadequate sample collection and manipulation or to poor analysis[296] although it is also well recognized that some of the disparities may be the result of biologic variations due to age, sex, pregnancy, physiologic conditions, dietary habits, environmental or occupational exposure, geographic circumstances or similar influences.[295] Values reported in 1962 for serum chromium are more than 3,000 times higher than the presently accepted values.[10] Additionally, the values reported in 1965 for 24-hour urinary chromium excretion are 750 times higher than the presently accepted values.[9] A critical study of the data compiled by Iyengar *et al.*[123] disclosed similar divergent values for a number of elements in various body fluids and tissues.[295]

Table II (trace element analyses of human serum)[261] and Table III (trace element analyses of human kidney (fresh))[261] further illustrate the range of elemental concentrations to be expected in human tissue analysis. The sensitivity requirements vary with these elemental concentrations, which in body fluids can range from about 10 μg/l reported for vanadium and 10 to 4,900 μg/l for silicon in urine analysis. Table IV is an additional compilation of the sensitivities

TABLE I

Reference values for chromium in human blood plasma or serum (Versieck[295]).

Investigators	Mean	SD (ng/ml)	Range	Analytical procedure
Kayne *et al.*[133]	0.14			AAS
Versieck *et al.*[297]	0.160	0.083	0.0382–0.351	NAA
Seeling *et al.*[240]			<0.50	AAS
Manthey and Kübler[166]	0.43	0.19	<1	AAS
Kasperek *et al.*[130]	0.45	0.15		NAA
Grafflage *et al.*[100]	0.73		0.23–1.90	AAS
Liu and Abernathy[159]	1.02	0.89		NAA
Rabinowitz *et al.*[226]	1.1		0.8–2.6	AAS
Pekarek *et al.*[215]	1.62	0.31	0.20–	AAS
Abraham *et al.*[1]	1.64[a]	0.925		AAS
	1.95[b]	1.007		
Liu and Morris[160]	1.67	1.74		NAA
van Kooten *et al.*[290]			0.7–3.2	NAA
Nakahara *et al.*[185]	2.14	11.4	3.0–44	NAA
Black *et al.*[26]	2.5			AAS
Hambidge[106]	3.1		1.0–	ESM
Davidson and Burt[58]	4.70	0.46	3.70–5.28	AAS
Davidson and Secrest[60]	5.07			AAS
Black and Sievers[25]	6.96		3.57–9.92	GC-MED
Salvadeo *et al.*[234]	8.2	2.4		AAS
Simenoff *et al.*[252]	8.51	3.88		PIXE
Kasperek *et al.*[131]	9.3	5.6		NAA
Behne and Diel[19]	10.3	6.2		NAA
Bierenbaum *et al.*[24]	12[c]	32		AAS
Savory *et al.*[236]	13.5		2.7–24	GC-ECD
Butt *et al.*[39]	15.5			ESM
Feldman[76]	17.4		5.1–40	AAS
Panteliadis[213]	20.5			ESM
Koch *et al.*[141]	22	12	7–52	ESM
Levine *et al.*[154]	23.2	4		AAS
Paixao and Yoe[212]	25		16–39	ESM
Glinsmann *et al.*[95]	28		23–34	AAS
Herring *et al.*[113,114]	28		9–56	ESM
Niedermeier and Griggs[190,191]	28	48	<10–260	ESM
Feldman *et al.*[77]	30		11–66	AAS
Bierenbaum *et al.*[24]	43[d]	113		AAS
Maxia *et al.*[168]	45		14–77	NAA
Freund *et al.*[86]			5–90	Unknown
Niedermeier *et al.*[192]	55		10–390	ESM
Li and Hercules[156]	150		41–251	CHL
Bala and Lifshits[15]	171			ESM
Monacelli *et al.*[177]	185		82–308	ESM
Nakahara *et al.*[186]	782	495	19.6–1460	NAA

[a]Men.

[b]Women.

[c]Subjects from Kansas City, Kansas.

[d]Subjects from Kansas City, Missouri.

Abbreviations: AAS—atomic absorption spectrometry; CHL—chemiluminescence; ESM—emission spectrometry; GC-ECD—gas chromatography electron capture detector; GC-MED—gas chromatography with microwave excited emission detector; NAA—neutron activation analysis; PIXE—proton induced X-ray emission.

TABLE II

Trace element analyses of human serum.

Element	$\bar{x}$ (mg/l)	No. of labs	Range (mg/l)	Techniques
Al	0.37	7	0.11–0.78	AAS, AES, MS, Chem.
As	0.043, 0.02	2	—	SAS, XRF
Ba	0.06	5	0.025–0.08	AES, MS, XRI
Be	—	—	—	—
Bi	<0.01	1	—	Elec
Cd	0.0059	3	0.0023–0.012	AAS
Co	0.01	10	0.00022–0.062	AAS, AES, NAA, Chem. Elec.
Cr	0.01	14	0.002–0.02	AES, NAA, Chem., Misc.
Cu	1.15	23	0.97–1.64	AAS, SES, NAA, XRF, Chem., Misc., Elec.
P	0.035, 0.019	2	—	Elec., Chem.
Fe	1.24	23	0.87–1.87	AAS, AES-FES, MS, NAA, Chem., XRF
Hg	0.012	1	—	NAA
I	0.07	5	0.045–0.100	Elec.
Li	0.01	4	0.008–0.027	AES-FES
Mn	0.013	16	0.00054–0.061	AAS, AES-FES, Elec., NAA, Chem.
Mo	0.017	4	0.006–0.027	AES-FES, NAA
Ni	0.033	8	0.0078–0.0580	AAS, AES-FES, XRI
Pb	0.12	4	0.016–0.13	AES-FES, XRF, Elec.
Sb	0.0032, 0.0025	2	—	NAA
Sc	0.15	8	0.098–0.327	NAA, XRF, Misc., Chem.
Si	4.3	5	2.5–10.0	AES-FES, Chem.
Sn	0.03, 0.03	2	—	AES-FES
Te	<0.04	1	—	XRF
Tl	<0.03	1	—	XRF
V	0.0046, <0.01	2	—	NAA, XRF
Zn	1.1	21	0.67–1.83	AAS, AES, MS, NAA, XRF, Chem., Elec.

Abbreviations: AES—arc emission spectrometry; NAA—neutron activation; Chem.—Chemical methods (e.g. colorimetry); MS—mass spectrometry; AAS—atomic absorption spectrometry; XRF—X-ray fluorescence spectrometry; AES-FES—arc emission-flame emission; Elec.—electrochemical methods; Misc.—miscellaneous.

TABLE III
Trace element analyses of human kidney (fresh).

Element	$\bar{x}$ (ppm)	No. of labs	Range (ppm)	Techniques
Al	0.7	5	0.35–1.80	AAS, MS, XRF
As	0.3, 0.35	2	—	MS, XRF
Bd	0.01, 0.01	2	—	MS, XRF
Be	—	—	—	—
Bi	0.4, 0.45	2		MS, XRF
Cd	19	4	12–33.1	AAS, AES, MS, NAA
Co	0.03	4	0.008–0.071	AAS, NAA, Chem.
Cr	0.3	3	0.03–0.86	MS, Chem., XRF
Cu	2.8	11	1.7–4.15	AAS, AES, XRF, MS, Chem., Misc., NAA
F	1	4	0.01–2.3	MS, Chem., XRF
Fe	78	4	42.100	AAS, NAA, XRF
Hg	0.43	6	0.063–2.75	AAS, AES, Chem., Misc.
I	0.04, 0.03	2	—	MS, XRF
Ia	0.01, 0.01	2	—	MS, XRF
Mn	0.6	7	0.4–2.4	Misc., NAA, XRF, AAS, AES, MS
Mo	0.63, 0.4	2		AES, MS
Ni	0.22, 6.0	2		AES, MS
Pb	0.9	9	0.27–1.27	AAS, AES, MS, Chem., XRF, Misc.
Sb	0.02	3	0.006–0.051	MS, NAA, XRF
Se	0.5	6	0.1–3.5	Misc., MS, NAA, Chem., XRF
Si	10.46	2	—	MS, XRF
Sn	0.3	4	0.2–0.48	AES, MS, XRF
Ie	5.8	3	0.57–16.03	AAS, NAA
Il	0.11, 0.003, <0.003	3	—	AES, MS, NAA
V	2.0, 0.007	2	—	MS, NAA
Zn	41	8	25.67	NAA, Chem., XRF, AAS, AES, MS

Abbreviations: As for Table II.

TABLE IV

Sensitivities and detection limits of analytical technique for some trace toxic elements.[a]

Element	Normal serum (ppm)[b]	ASV detection limits (ppm)[c]	AAS sensitivity (ppm)[d]	ETA-AAS sensitivity (ppm)[e]	DC arc ES detection limits (ppm)[f]	ICP-ES detection limits (ppm)[g]	NAA sensitivity (ppm)[h]	SSMA detection limits (ppm)[i]	XRF detection limits (ppm)[j]
As	0.19	—	0.1	0.002	10	0.002	0.05	0.0006	—
Be	<0.004	—	0.02	0.000001	0.05	0.0004	—	0.00008	1
Cd	0.005	0.000005	0.01	0.000003	2	0.00006	0.005	0.007	2.5
Hg	0.003	0.001	2.2	0.0005	2	0.001	0.003	0.007	1
Cr	0.0028	—	0.06	0.00004	1	0.00008	0.3	0.0005	0.7
Ni	0.03	—	0.07	0.0001	0.2	0.0002	0.7	0.002	0.2
Se	0.011	—	0.5	0.0001	100	0.03	0.01	0.002	—

[a]Adapted from Morrison[182]; [b]Altman and Dittmer[8]; [c]Dulka and Risby[69], Copeland and Skogerboe[54]; [d]Varian Techtron Ltd.[291]; [e]Kahn *et al.*[129]; [f]Koch and Koch-Dedig[140]; [g]Fassel and Knisely[74], Fassel[73]; [h]General Activation Analysis[93]; [i]Hamilton *et al.*[108]; [j]Koch and Koch-Dedic[140], Hamilton *et al.*[108].

Abbreviations: ASV—anodic stripping voltametry; AAS—atomic absorption spectrometry; ETA-AAS—electrothermal automation-AAS; ES—emission spectroscopy; ICP-ES—inductively coupled plasma emission spectrometry; NAA—neutron activation analysis; SSMS—spark source mass spectrometry; XRF—X-ray fluorescence spectrometry.

and detection limits of six of the most frequently cited techniques employed for the determination of arsenic, beryllium, cadmium, chromium, nickel and selenium.[261]

From the above analytical considerations, one can concur with the admonitions and analysis of Versiek[295] that mounting evidence would suggest that much previous work on trace elements in human body fluids and tissues must have severely suffered from methodological deficiencies. It is to be especially stressed that trace element analysis is affected by major potential problems at the sample collection and measurement stage. Sample contamination is generally acknowledged to be a principal source of error when the concentrations decrease to the nanogram or subnanogram level, e.g., arsenic, cadmium, chromium, nickel, cobalt, manganese, aluminum in serum with large errors being introduced by working in an uncontrolled environment by employing containers and laboratory devices that are not especially selected and meticulously cleaned.[295]

MULTIELEMENT DETERMINATIONS

The need for monitoring of toxic and essential trace elements in biological materials has led to an increasing demand for suitably sensitive and selective analytical techniques with multielement capabilities. A number of specialized techniques (some of which are relatively expensive) have been successfully applied for this purpose. The principal examples include: (1) SIMAAS (simultaneous multielement atomic absorption spectrometry; (2) atomic emission spectrometry with inductively coupled plasma excitation (AES-ICP); (3) electrothermal atomic absorption spectrometry (ETA-AAS); (4) spark source mass spectrometry; (5) X-ray fluorescence (XRF); (6) neutron activation analysis (NAA); and (7) anodic stripping voltametry.[2,130,182,245,261,297]

a) Inductively coupled plasma-atomic emission spectrometry (ICP-AES)

The argon supported inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) (introduced in the late 1970s) is rapidly becoming an accepted analytical tool, and is being increas-

ingly employed for the characterization of metals in environmental and biological samples. This increasing utility can be attributed to its unique features which include: (a) simultaneous multielement capability; (b) working range of 4–6 orders of magnitude (Table IV) permitting the simultaneous determination of major, minor and trace elements under a single set of operating conditions and a single sample preparation procedure; (c) minimal background and matrix interferences; and (d) remarkable stability of the plasma vaporization-atomization excitation source.

The ICP excitation source is an electrodeless argon plasma at atmospheric pressure and maintained by inductive coupling to a radio frequency electromagnetic field. Because argon is initially neutral and non-conducting, the plasma must be initiated by "seed electrons" which are usually generated by a brief Tesla discharge. The plasma forms immediately and is self-sustaining after the initial formation. The highly ionized gas that results has temperatures of 6,000–10,000 K. The ICP temperature is higher than that of typical flames and graphite furnaces (2,000–4,000 K), slightly higher than DC arcs (4,000–7,000 K) and lower than sparks (10,000 K).[245] Precisions of about 5% have been achieved with the ICP-AES system. The cost of the instrumentation in the late 1970s was in the range of $60,000 to $80,000.[58,74,103,138,182,266,284]

Subramanian and Meranger[267] recently determined 20 elements (including As, Be, Cd, Cr, Ni, Hg and Se) in 143 autopsied liver and kidney (cortex and medulla) specimens from two Ontario, Canada communities using both ICP-AES and electro-thermal atomization-atomic absorption spectrometry coupled with hydride evolution (As, Se). Table V gives the detection limit, sensitivity and wavelength for each of the elements determined in the tissue samples. The detection limits are given in mg/l and correspond to 3SD of baseline noise. The detection limit value for each element was sufficiently low to permit its determination in the tissue samples of even normal individuals. As noted in Table V the analytical techniques used for the various elements were sensitive down to the μg/l level. The ICP-AES linear dynamic range was 0–1,000 mg/l for all the elements listed in Table V (except for Ca and Mg with linear ranges only up to 100 and 20 mg/l). It is this wide dynamic range of the ICP-AES that permits the simultaneous determination of several major, minor

TABLE V

Analytical lines and detection limits.

Element	Wavelength (nm)	Detection limit[a] (mg/l)	Sensitivity[b]
Ag	328.07	2.00	0.002
Ba	455.40	0.01	0.006
Be	313.04	0.001	0.0001
Ca	315.89	0.07	0.007
Cd	226.50	0.002	0.0005
Co	345.35	0.05	0.01
Cr	283.56	0.008	0.002
Cu	324.75	0.008	0.0008
Fe	259.94	0.20	0.02
K	766.49	1.0	0.1
Mg	279.5	0.04	0.02
Mn	257.61	0.01	0.0003
Mo	379.83	0.30	0.02
Na	588.995	2.0	0.001
Ni	231.60	0.01	0.004
P	214.91	0.60	0.1
Sr	421.55	0.005	0.002
Th	401.91	0.06	0.002
Ti	337.28	0.01	0.009
Zn	202.55	0.02	0.004
As	193.70	0.001	0.001
Se	196.00	0.001	0.001
Pb	217.00	0.01	0.04
Hg	253.70	0.0002	0.001

[a]Concentration of the element in mg/l which will produce a net line intensity equal to 2 SD of blank with a minimum of 10 successive measurements. To convert the detection limit in mg/kg of wet tissue multiply the above values by 10.

[b]The sensitivity for ICP-AES elements (Ag to Zn) is given in units of (mg/l)/mV; for As, Se, Pb, and Hg it is given in (mg/l)/0.0044 absorbance.

and trace elements using a single wet digestion procedure, a single set of operating conditions and a single set of calibration curves. The linear ranges in mg/ml for As, Hg, Pb and Se were 0–0.06, 0–0.02, 0–1 and 0–0.07 respectively.

The reliability of the wet digestion-ICP-AES technique was ascertained by analyzing NBS bovine liver (SRM1577). As Table VI illustrates, the observed values agree well with the certified data

TABLE VI
Accuracy and precision of ICP-AES for analysis of biological materials: NBS bovine liver.

Element	Observed value (mg/kg)[a]	±SD[b]	Certified value (mg/kg)
Ag	<2.00	—	0.1
Ba	0.10	0.04	—
Be	<0.01	—	—
Ca	118	5	123
Cd	0.26	0.03	0.27
Co	0.20	0.05	0.5
Cr	0.40	0.16	—
Cu	180	6	193
Fe	263	6	270
K	9326	410	9700
Mg	648	30	605
Mn	9.5	0.2	10.2
Mo	6	1	3
Na	2234	159	2430
Ni	0.33	0.16	—
P	11,121	1,134	11,000
Sr	<0.05	—	0.14
Th	0.06	—	—
Ti	<0.01		—
Zn	120	4	130

[a]In dry weight of sample.
[b]Calculated from 14–16 determinations, each consisting of three 10s integrations.

attesting to the validity of using the same digestion procedure and the same set of operating conditions for the various elements of interest.[266]

Inductively coupled plasma (ICP) has some advantage over the traditional excitation sources including combustion flames, arcs and sparks. Flames are limited by relatively low temperature, making it difficult to analyze refractory elements or elements with high excitation energies, especially at low concentration levels. Additionally combustion products and flame gases can give rise to both chemical and spectral interferences. In contrast, the ICP excitation source is

an electrodeless argon plasma at atmospheric pressure and maintained by inductive coupling to a radiofrequency electromagnetic field. The high temperature used results in greater freedom from chemical interferences and increases the range of elements that can be determined.[182,245]

Presently available ICP spectrometers (second generation sequential ICP spectrometers) have roughly comparable detection limits demonstrated by the earlier models, and throughputs ranging from 20 to 50 elemental determinations per minute. Additionally, many models are fully automated. New developments in the field of IC emissions such as operation with electrothermal vaporization accessories, low flow torches and significant advances in radiofrequency (RF) power technology are incorporated in the new designs. Many manufacturers have developed appropriate peak finding algorithms, wavelength/accuracy/repeatability specifications and calibration schemes for completely automated operation of the monochromator.[63]

It is important to note a number of additional recent references dealing with the utility of the ICP-AES technique. These include its utility for the determination of trace elements in blood and urine,[17,62,183,318] bone,[164] hair[169,318] and miscellaneous biological tissues.

Additionally it is important to cite recent advances dealing with sample introduction prior to multi-element determination by ICP-AES. For example, pneumatic nebulization appears to be the most popular method of sample introduction although the sensitivity attainable is not sufficient for the ICP analysis of many elements which are in the nanogram per gram range.[29] A number of studies have concentrated on developing methods for isolating trace elements from complex mixtures including coprecipitation, chelation,[17,176] chromatography,[41] and conversion into hybrides.[308] Ultrasonic nebulizers have demonstrated improvements in working ranges by factors of 1.1–12.[20,280]

Habib and Salin[104] recently described the application of controlled potential electrolysis with both graphic electrodes and a hanging mercury drop electrode as separation and preconcentration techniques for ICP-AES using a direct sample insertion device (DSID) for the determination of heavy metal ions in solution. With a deposition time of 5 minutes, the detection limits for Cd, Ni, Co, Cu, Pb and Zn were 175, 25, 2.59, 2.4 and 680 ng/ml, respectively.

A computer-controlled graphite direct insertion device for direct analysis of plant samples by ICP-AES has also been recently reported.

In summary of the advantages of the ICP-AES, the general detection limits for most elements are in the range of 0.1–10 ppb, good accuracy and precision have been observed and the analysis time is rapid. Forty or more elements can be measured in several minutes using a direct-reading computer-controlled spectrometer with the attainment of both good accuracy and precision. The major disadvantages include the expense of the equipment and the requirement of a skilled operator.[245]

b) Proton-induced X-ray emission analysis (PIXE)

The use of nuclear particle accelerators has generated a variety of techniques such as PIXE which is being increasingly employed for multiple trace element analysis in matrices such as hair and biological tissues.

The determination of trace elements in blood and urine as a measure of exposure is standard practice. However, hair may also be useful as its collection is non-invasive, it can be stored and its analysis for trace elements can be readily accomplished with recognized precautions. Additionally, the concentration of many trace elements is much higher in hair than in blood or urine and hair may thus provide a ready record of a much longer period of exposure to heavy metals than either. Trace elements such as Ni, Pb, Mn, Fe, Zn, K, Ca, Br, Rb and Sr can be routinely measured in hair in most samples from occupationally exposed workers and Cr, As, Se, Cd, Co, Bi and Zr can be measured less frequently.[48] In the study of Clayton and Wooler, PIXE spectra were measured by using a 2.5 MeV proton beam in vacuum with currents between 30 and 40 nA (beam size 2 mm). An accumulated charge of 50 μC was collected, taking some 20–25 min per sample. X-rays were detected with a 4 mm diameter Si (Li) detector. The X-ray spectra were analyzed off-line by using the programs of Clayton *et al*[48] Other studies describing the utility of PIXE analysis include its utility for the determination of trace elements (Ni, Fe, Zn) in human urine,[85] human serum,[163] and miscellaneous biological samples.[16,21,35,136,153,157,302]

c) Voltammetric techniques

Voltammetric techniques such as anodic stripping voltammetry (ASV), cathodic stripping voltametry (CSV), differential pulse polography (DPP) and the more recent approach of absorption voltammetry (AV) are electrochemical techniques that are finding wide application for measurement of trace and ultra trace quantities of metals in solution. ASV is an extremely sensitive technique which can determine ultra traces of metals present at 10^{-9} to 10^{-10} M even in samples with high concentrations of calcium and sodium ions. Although the sensitivity of ASV is somewhat greater than AAS, the range of metals that can be detected is more limited. Although ASV is a very matrix-sensitive technique and there has been a lack of standardized procedures in the past, simultaneous determinations of usually from 4 to 6 elements can often be done with no additional plating time, equipment or sample requirements. The price of a complete and versatile ASV system including cell and electrodes appear to be relatively modest (*ca.* $5,000). Typical analysis time in ASV is about 10 minutes, with accuracy and precision on the order of 3 to 10% are obtained for typical trace concentrations of 10^{-5} to 10^{-8} M.[182,245]

The greatest disadvantages of the ASV technique include: the lack of standard procedures, the relatively limited number of metals that can be analyzed, long deposition times, the potential problem of a metal in the bound form being undetected, and the requisite high level of skill required for optimal operation.[182,245,261]

Although the voltammetric techniques, particularly ASV, have become more widely accepted for the determination of trace elements in various matrices, the *optimum* capability of the voltametric approach for multi-element determinations is not *generally* being accomplished.[4,182]

One of the major factors influencing the accuracy of the proposed scheme of Adeloju *et al.*[4] in determining 7 to 8 elements in a single biological sample digest is the chosen decomposition method. Decomposition by wet digestion methods are preferable to the dry ashing methods for this proposed scheme. However, the authors advise that careful precautions still have to be taken to ensure both adequate decomposition of the organic matrix and retention of the analyzers in states or forms amenable for optimum detection and hence accurate determination.

Adeloju *et al.*[4] developed a simple and reliable voltammetric approach for multi-element determination of up to eight elements in a single biological sample digest at major, minor, trace and/or ultra trace levels. The method employs a wide range of voltammetric techniques such as polarography, cathodic stripping, anodic stripping, and absorption voltametry in their differential pulse mode together with some chemical manipulations for sequential simultaneous determination of cadmium, selenium, nickel, lead, copper, zinc and cobalt in the same solution. Arsenic can also be determined under favorable conditions. The determination of the eight elements in the digested samples takes about 3 hours or approximately 25 minutes for each element per sample. An analytical scheme for the sequential simultaneous determination of Se, Cd, Pb, Cu, Zn, Ni and Co in a single sample solution by differential pulse voltammetry is reported in Reference 4. Selenium can be readily determined at concentrations $>0.1\ \mu g/l$ following the sample decomposition. Typically the concentration of Cd in biological materials is often relatively lower than those of the other three elements. Hence, its accurate determination requires an individual determination using DPASV with a long preconcentration time (10–30 min) at a disposition potential of -0.8 V *vs* Ag/AgCl (saturated KCL). Table VII depicts the application of the proposed multi-element approach to the determinations of these metals in various biological materials. Table VIII shows the total analysis time for a range of multi-element determinations by the proposed scheme.

The scheme proposed by Adeloju *et al.*[4] in their study for multi-element determination not only is unique for its ability to extend the number and range of elements that can be determined in a single sample solution, but also provides a link with the new generation of voltammetric analyzers for complete automation of voltammetric trace analysis.

SINGLE-ELEMENT TECHNIQUES

Electrothermal atomization-atomic absorption spectrometry (ETA-AAS)

It is generally acknowledged that the fastest growing technique in its application to the analysis of trace elements in biological samples is

TABLE VII

Application of the proposed multielement approach to the determination of some trace elements in various biological materials.

Element	Animal muscle[a] (μg/g)	Bovine liver[a] (μg/g)	Orchard leaves[a] (μg/g)	Urine[a] (μg/L)
Se	0.254 ± 0.003[b] (0.28 ± 0.03)			23.6 ± 0.3[a]
Cd	0.128 ± 0.005[c]		0.10 ± 0.01[c] (0.11 ± 0.01)	0.57 ± 0.03[c]
Pb	0.35 ± 0.01[c]		39 ± 1[c] (45 ± 3)	129 ± 1[c] (115 ± 35)
Cu	3.68 ± 0.07[c] (3.96 ± 0.33)	194 ± 3[d] (193 ± 10)	13.1 ± 0.4[c] (12 ± 1)	204 ± 4[c] (174 ± 60)
Zn	86 ± 1[c] (86.3 ± 3.4)	137 ± 2[d] (130 ± 13)		1840 ± 10[c] (1500 ± 500)
Ni		0.20 ± 0.01[e]	1.32 ± 0.02[e] (1.3 ± 0.2)	32.9 ± 0.5[e]
Co		0.16 ± 0.01[e]	0.105 ± 0.002[e]	0.55 ± 0.01[e]

[a]Error is mean deviation for triplicate determination. Certified values and manufacturer assay data for urine are in parentheses; error is standard deviation based on results from various instrumental techniques. [b]By DPCSV. [c]By DPASV. [d]By DPP. [e]By DPAV.

TABLE VIII

Total analysis time for a range of multielement determinations by the proposed scheme.

Elements determined sequentially	No. of elements	Range of techniques utilized	Analysis time[a] (min)
Se, Cu	2	DPCSV, DPP	55
Cu, Ni	2	DPP, DPAV	30
Se, Cu, Zn	3	DPCSV, DPP	65
Se, Co, Ni	3	DPCSV, DPAV	70
Cu, Co, Ni	3	DPP, DPAV	40
Se, Cu, Co, Ni	4	DPCSV, DPP, DPAV	80
Cu, Zn, Co, Ni	4	DPP, DPAV	50
Se, Cd, Pb, Cu	4	DPCSV, DPSAV, DPP	90–115[b]
Cd, Pb, Cu, Ni, Co	5	DPASV, DPP, DPAV	90–115[b]
Se, Cd, Pb, Cu, Zn	5	DPCSV, DPASV, CPP	105–130[b]
Se, Cd, Pb, Cu, Ni, Co	6	DPCSV, DPASV, DPP, DPAV	120–145[b]
Cd, Pb, Cu, Zn, Ni, Co	6	DPASV, DPP, DPAV	105–130[b]
Se, Cd, Pb, Cu, Zn, Ni, Co	7	DPCSV, DPASV, DPP, DPAV	135–160[b]
As, Cd, Pb, Cu, Zn, Ni, Co	7	DPCSV, DPASV, DPP, DPAV	135–160[b]

[a]Includes deoxygenation time and two standard additions.

[b]Depends on concentration of cadmium in sample; analysis time can be more if $\ll 1$ μg/L or less if > 5 μg/L.

ETA-AAS whereby electrically heated graphite tubes or rods reaching temperatures up to 3,000 K replace the regular flame in this modification of AAS.[182,245,261] Similarly to AAS, ETA-AAS is a single-element technique and is specific for the element being determined. The major advantages of ETA-AAS over standard AAS are small sample volume (e.g., 5–10 μl) compared to several ml needed for aspiration in AAS) and high sensitivity (100 fold or more improved over AAS) (Table IV). However, it should be noted that a number of interferences can plague the technique (e.g., requirement for ultra cleanliness, finite lifetimes of graphite rods, etc.). The precision of ETA-AAS is normally slightly less than for flame AAS, on the order of 2 to 5%.[182]

The applications of ETA-AAS in biological analysis has been burgeoning, especially for the determination of essential and toxic trace elements in blood and serum.[182,245,261]

It should be noted that in AAS and ETA-AAS a background corrector is required to avoid absorbing light from other material that can absorb light at the element wavelength. Although the deuterium arc system is the most common type of background corrector currently employed, another type of correction can be done by a Zeeman background corrector. In the Zeeman type correction which is being increasingly employed, correction is done at the exact wavelength of the element being measured, and background does not usually affect absorption. However, one disadvantage of Zeeman correction is that sensitivity is reduced by 10–50% depending on the element.[245] Recent applications of Zeeman AAS for the determination of metals in biological samples include its utility for the direct determination of heavy metals (Cd, Ni, Cr, Hg, Pb) in blood and urine,[28,148] the determination of Cd, Pb, Cu and Mn in human kidney cortex,[221] and Cd, Se, Zn, Cu, Pb and Fe in human seminal plasma and spermatozoa.[220]

Although ETA/AAS has been utilized in single element determination it should be noted that the determination of metals at the microgram/liter level in blood serum by simultaneous multi-element atomic absorption spectrometry (SIMAAC) with graphite furnace atomization has been accomplished recently.[155] For example, seven metals, Ci, Ni, Co, Mn, Mo, V and AL, have been determined in blood serum with this technique.

DETERMINATION OF INDIVIDUAL METALS

a) Nickel

Nickel has been determined in biological materials such as human blood and urine by a variety of techniques including atomic absorption spectroscopy,[67,201,237,241,259,304,316] voltammetry,[3,89,218] spectrophotometry,[50,212,271] emission spectrometry,[113,141,177,190] optical emission spectrometry with an inductively coupled plasma source X-ray fluorescence.[97] Additionally nickel has been determined in human milk by electrothermal atomisation atomic absorption.[230]

The accuracy and precision of analyses for nickel in body fluids have gradually improved over the last two decades and the detection limits for nickel have steadily declined.[32,260,272] For example, it is currently feasible to measure serum nickel concentrations in 1-ml samples[272] contrasted with 10-ml samples which were required in 1970.[274] Additionally, the techniques for collecting specimens and avoiding nickel contamination during sample processing have also been substantially refined.[32,152,260,272,273] Hence, reference values for nickel concentrations in sera of healthy persons have diminished from 2.6 (SD 0.8) μg/l in 1970[274] to 0.46 (SD 0.26) μg/l in 1984.[273]

It is important to cite a recent study of Leach *et al.*[152] in which the incidence, magnitude, and time-course of hypernickelemia in patients with acute myocardial infarction were examined utilizing stringent clinical criteria, up-to-date analytical instrumentation and utmost precautions to minimize contamination of samples with nickel. Nickel was measured by electrothermal atomic absorption spectrometry,[273] employing a Zeeman background correction system, in sera from (a) 30 healthy adults, (b) 54 patients with acute myocardial infarction, (c) 33 patients with unstable angina pectoris without infarction, and (d) 5 patients with coronary altherosclerosis. Employing the ETA-AES technique of Sunderman *et al.*[273] the detection limit for nickel was 0.05 μg/l; coefficients of variation for replicate analyses averaged 3.8% (within-run) and 8.1% (day-to-day). Analytical recovery of nickel, added to 16 sera samples to give a concentration of 8 μg/l averaged 97% (SD $\pm$3%), while analytical recovery of nickel similarly added to 13 samples of whole blood averaged 103% (SD $\pm$6%). Nickel concentrations in 30 sera samples measured by Sunderman *et al.*[273] did not differ significantly ($r = 0.980$) from results obtained by the IUPAC Reference Method.[32] Nickel

was also measured in urine specimens by ETA-AAS, after acid digestion, chelation and solvent extraction, utilizing the IUPAC Reference Method.[32] In the above study of Leach *et al.*[152] serum nickel concentrations were found to be unrelated to age, sex, time of day, cigarette smoking, medications, clinical complications or outcome. The mean and (SD) nickel concentrations in Group A (healthy adults) were 0.3 μg/l (range <0.05–1.1 μg/l). Within 72 hours after hospital admission, hypernickelemia (Ni ≧1.2 μg/l) was found in 41 patients of group B (76%) and 16 patients of group C (48%).

The IUPAC Reference Method for the analysis of nickel in serum and urine by ETA-AAS[32] involves the initial digestion of urine or serum samples with nitric-, sulfuric- and perchloric acids, the adjustment of the solution to pH 7 with ammonium hydroxide. Following the addition of ammonium tetramethylene-dithiocarbamate, the bis(1-pyrrolidine carbodithioato)nickel) (II) complex is extracted with methylisobutylketone and the nickel determined by ETA-AAS. Employing this technique, an interlaboratory comparison showed mean Ni levels in pooled serum and urine of 12.7 and 8.3 μg/l respectively, with interlaboratory coefficients of variation of 24 and 23% respectively. Mean within-run and run-to-run coefficients of variation were 10.6 and 12.4% for serum and urine respectively. It was advised that the above technique should not be employed to determine Ni in whole blood or tissues.[32]

The voltammetric technique of Pihlar *et al.*[218] for the determination of Ni (and Co) in biological materials (and food and water) consisted of the application of d.c. or differential pulse voltammetry after prior interfacial accumulation of absorption layer of dimethylglyoximate at the hanging Hg drop electrode. In aqueous media the detection limits of 1 nanogram/l were obtainable for Ni (II) with good precision and accuracy.

b) Cadmium

The human health effects of exposure to cadmium have been extensively reviewed recently.[51] The main feature of cadmium metabolism is its very long biological half-life (up to 30 years), and hence its progressive accumulation in the organism, with no metabolic-pattern allowing its elimination from the organism. While metallothionein, an intra-cellular cadmium-binding protein corresponds to a

defense mechanism, it is also responsible for a selective accumulation of Cd in the kidney. General aspects of biological monitoring have been reviewed by Lauwerys.[150] In non-occupationally exposed adults, Cd levels in blood are usually below 1 μg/100 ml whole blood. Over 70% of the Cd is bound to red cells in blood. Cadmium accumulates mainly in the kidney and liver with about 50% of the body burden found in these organs. Cadmium is bound in all tissues to metallothionein, and is principally excreted via the urine and in adults not occupationally exposed to Cd, the level in urine is usually below 2 μg Cd/g creatinine, and the extent of urinary excretion increases with age.[150] Since in urine, Cd is present mainly bound to metallothionein, the determination of the level of this protein in urine may provide the same information as the determination of Cd *per se*. Additionally, metallothionein analysis presents an advantage over direct Cd analysis since it is not subject to external contamination and a sensitive radioimmunoassay has been developed for its determination in urine.[42,43,282,283]

Cadmium in blood has been shown to reflect mainly the last few months exposure in workers and a whole value of 1 μg Cd/100 ml whole blood was proposed as a tentative no effect level for long-term exposure.[150]

A survey of the analytical methods utilized for cadmium detection has been earlier described by Smiley and Kessler[255] and Stoeppler.[263] The principal analytical procedures for the determination of cadmium in urine on blood include: (a) electrothermal atomic absorption spectropathometry;[61,68,75,112,146,179,246,258,264,268,301] (b) Zeeman-AAS.[6,7,28,47,135,148,250,350] Less frequently employed procedures include: (a) spectrophotometric;[38] (b) anodic stripping voltammetry;[52,124,134,194,311,317] (c) inductively coupled plasma-atomic absorption spectroscopy (ICP-AES);[199] and (d) GC and HPLC following liquid–liquid extraction with diethyldithiocarbamate.[65,66]

The determination of Cd from tissues (e.g., kidney, liver, pancreas, placenta) has also been principally accomplished by AAS,[146,193,286] Zeeman-AAS,[221] and differential pulse anodic stripping voltammetry.[225]

Most earlier published methods required extraction of Cd from the biological matrix[207] and only a few direct determinations of low levels of Cd in blood and urine have been reported.[31,222]

Sample contaminations and matrix effects appear to cause significant problems in the accurate determination of submicrogram levels of Cd in biological matrices.[80,137,255,263] Because of the ultra trace levels of Cd (e.g., less than 1 μg/l in urine and less than 3 μg/l in blood) found in the normal population (non-occupationally exposed to Cd), it is desirable to have a direct *in situ* method for AAS analysis of Cd. For example, Subramanian *et al.*[268] described a graphite furnace AAS technique with matrix modification for the determination of nanogram per milliliter levels of Cd in human urine. The procedure utilized a diammonium hydrogenphosphate-nitric acid, an ammonium nitrate-nitric acid matrix modifier addition to the urine sample prior to determination. It was suggested that the detection limits were sufficiently low for screening programs.

In the procedure of Stoeppler and Brandt[264] for the determination of Cd in whole blood and urine by ETA-AAS, 50 to 200 μl aliquots of whole blood were treated with IM HNO_3 for deproteinization and matrix modification. After centrifuging, the supernatant was analyzed by automated ETA-AAS. The precision expressed as day-to-day precision varied from 30% to 0.4 μg Cd/l to 3.8% at 9.3 μg Cd/l whole blood. If 25 μl of a 1+3 diluted blood sample are injected, a detection limit of $\leqq$0.2 μg Cd/l was achieved.

A semi-automated procedure was developed by Watanabe *et al.*[301] for the determination of Cd in blood by utilizing a combination of block-digestion-autosampler-flameless AAS. For block-digestion which can mineralize 144 samples in 10 hrs, a special micro Kjeldahl tube of special shape was utilized. When applied to the mass analyzer of Cd in 1,761 blood samples from non-exposed individuals, the system as a whole possessed sufficient sensitivity with the lowest limit of detection of 0.2 nanograms Cd/ml blood. Fifty samples of Cd in blood per day could be analyzed by this procedure.

A comparative determination of Cd in blood by four different techniques was described by Alt.[6] In three of the methods, the final step of determination involved graphite furnace AAS and the fourth, the anodic stripping voltammetry was used with a rotating glass electrode and enrichment on a Hg film. In the first method a direct determination without matrix separation was accomplished by mixing blood with 2-propanol and Lumatom (2 comm. qt. NH.OH reagent) and pippetting the mixture into a Zeeman-AAS instrument. Relative standard deviation was 0.09 at 2.2 m μg Cd/l. In the second

method, the AAS determination was preceded by precipitation of the protein matrix with 1MHNO_3. In methods 3 and 4, the organic matrix was destroyed by an oxidizing digestion with HNO_3—H_2SO_4-perchloric acid, followed in method 3 by extraction with trioctylamine in methylisobutylketone. The difficulties in measuring the background absorption in the direct method were overcome by Zeeman compensation (Zeeman-AAS). All methods were found to be equally reliable and gave a satisfactory recovery of 2.0–11.9 m μg Cd/l.

The NIOSH procedure (P&CAM233)[194] for the determination of Cd in blood involves an initial wet-ashing of a sample of whole blood with a mixture of nitric, perchloric and sulfuric acid, dissolving the residue in an acetate buffer solution and analyzing the buffered sample by anodic stripping voltammetry with a composite mercury-graphite electrode. The detection limit is 2 nanograms which corresponds to a 0.002 μg/ml in a 1-ml sample. The linear range is 0.002 to 1 μg/ml in 1 ml of blood. The precision (CV) of the method is 0.05 at 0.05 μg/ml.

As noted earlier, a large part of the blood Cd is related to recent exposure. Under long term, low-level exposure, the concentration of Cd in blood is thus a useful indicator of the exposure to Cd in recent months. The half-time of Cd in blood is estimated to be 2–3 months[37] if the exposure levels do not undergo major changes.[305]

The relationship between the body burden of Cd and indirect biological indices of exposure (e.g., urine, blood and hair) was evaluated by Ellis *et al.*[70] employing graphite furnace AAS. The detection limits were 1 mg/l for urine, 0.1 μg/dl for blood, and 0.5 μg/g for hair. It was found that in general, blood and urine Cd levels are useful only as *indices* of significant exposure. At present, direct *in vivo* measurements of kidney and liver Cd are suggested to provide the most accurate data on body burden of Cd as well as the best indices of accumulative exposure.

The advantages of a clean-up procedure for the determination of Cd in urine by ETA-AAS were described by Heinrich and Angerer.[112] To urine samples were added buffer, distilled water and hexamethylene ammonium-hexamethylene dithiocarbamate in disopropylketone-xylene (70:30). After mixing and centrifuging, the organic phases were analyzed at 228.8 mm with 2H arc source background correction. The detection limit was <0.3 m μg Cd/l;

recovery was 93–11% and the standard plot was linear up to 10 m μg/l.

c) Chromium

Although chromium is considered an essential trace element for humans, there is recognized concern for the potential environmental sources and hazards of exposure to chromium. Numerous studies have indicated the importance of the determination of specific chemical states of environmental chromium.[72,79,80,88,149,187,203,227] Although Cr in inorganic compounds may occur in valence states ranging from -2 to $+6$, the Cr(III) and Cr(VI) species are of primary environmental interest. Compounds containing Cr^{6+} present largely from anthropogenic processes are believed to be responsible for most of the health problems associated with all Cr compounds. The toxicity and carcinogenicity of chromium compounds is largely associated with the Cr(VI) form. Hence, the speciation of hexavalent Cr and the trivalent form is of great interest since Cr(VI) is a potentially toxic substance while Cr(III) is an essential trace element for man and animals.

In view of the above, the separation and determination of chromium at trace levels has received considerable attention. It is widely recognized that the assessment of Cr status by direct measurements of Cr in specific tissues and body fluids has been exceptionally difficult. The analytical methodologies for the determination of Cr in biological materials is only recently attaining a level of acceptance such that interlaboratory values for total Cr in biological materials can be evaluated.[10] As noted earlier (Table I), absolute values for Cr in biological materials reported earlier varied considerably and even within the same laboratory, as further illustrated in Table IX which shows a number of reported concentrations of Cr in blood from the period 1948–1974.[171] Values reported in 1962 for serum Cr are more than 3,000 times higher than the presently accepted values (*ca.* 0.8–1 ppb) and the values reported in 1965 for 24-hour urinary Cr excretion are 750 times higher than the presently accepted values.[9]

Determination of chromium in urine and plasma has been the subject of much effort recently[10,18,111,115,133,175,253,292,296] but published reference intervals for concentrations of Cr in plasma and

TABLE IX

Reported chromium concentrations in blood.

Authors	Year	Concentration (μg/liter)
Grushko, Y. M. (*Biokhimiya* 13: 124)	1948	35
Urone, P. F., *et al.* (*Anal. Chem.* 22: 1317)	1950	50
Monacelli, R., *et al.*[177]	1956	180
Volod'ko, L. V., *et al.* (*Vestn. Akad. Nauk. Belarusk* 1: 107)	1962	200
Schroeder, H. A., *et al.* (*J. Chronic Dis.* 15: 941)	1962	520; 170
Wolstenholme, N. A. (*Nature* 203: 1284)	1964	1,000[a]
Feldman, F. J., *et al.*[77]	1967	29
Hambidge, K. M. (In: *Newer Trace Elements in Nutrition*, Dekker, NY)	1971	7
Cary, E. E., *et al.* (*J. Agri. Food Chem.* 19: 398)	1971	7
Davidson, I. W. F., *et al.*[59]	1973	4.7
Pekarek, R. S., *et al.* (*Anal. Biochem* 59: 283)	1974	0.72

[a]Calculated on the basis of 22.4% total solids in blood.

urine still vary widely.[296] The main obstacles to accurate Cr measurement are acknowledged to include: (a) poor sample handling techniques giving rise to samples and reagent contamination; (b) the low concentrations of Cr in plasma and urine which requires considerable analytical sensitivity; and (c) the interference problems in several sensitive AAS methods, caused by inadequate background correction around the spectral line of Cr.[18,60,101,133,233,281,292] Formerly, a deuterium arc lamp (190–350 mm) was used as a background corrector source, but recently the use of a tungsten/halogen lamp (350–900 mm) has greatly improved correction around the chromium line.[133,292] Thus, generally prior to 1978, numerous erroneous results were reported for Cr in urine using ETA-AAS because of the inability of *conventional* systems to correct for the high nonspecific background absorption. Reliable results for Cr in

urine, 0.1–0.3 nanograms/ml[101] were first determined by using a continuum source, echelle monochromator, wavelength-modulated AAS (CEWM-AAS).[110] The combination of a continuous source and wavelength modulation offers CEWM-AAS background correction capabilities superior to conventional AAS, with the exception of Zeeman AAS, without a significant loss in detecting power.[110,111] The CEWM-AAS results were confirmed by using stable isotope gas chromatography/mass spectrometry (SID/GC/MS).[293]

Morris and Kemp[181] recently described the utility of ETA-AAS for directly measuring Cr concentrations in plasma and urine employing tungsten/halogen background correction, automatic sample injection, and the use of pyrolytically coated graphite tubes, combined with stringent sample-handling procedures. All AAS analyses were performed at a wavelength of 359.9 mm, a spectral band width of 0.7 mm, and a lamp current of 20 mA, using a tungsten/halogen hollow-cathode lamp as the background corrector. The standard-additions procedure produced a linear response up to at least 3 μg/l added chromium. Analytical recovery of Cr added to plasma at three concentrations (0.56, 1.08 and 2.72 μg/l) was 100%, 99% and 94%, respectively ($n=2$ each). The sensitivity, defined as the amount of Cr giving 10% absorbance, was 4.6 pg for a 30 μl sample (0.15 μg/l); the detection limit, defined as the amount of Cr giving an absorbance corresponding to twice the standard deviation of the blank, was 2.3 picograms (0.08 μg/l). In blood and urine samples from 47 healthy volunteers (ages 18–79 years), the variances and the means of Cr concentrations were not significantly sex-related ($p=0.05$). The combined "normal" mean (± 250) for plasma Cr was 0.82 ± 0.52 μg/l, and for urine Cr, <1.0 μg/l no Cr was detectable in the urine samples from six of three subjets.[181]

Atomic-absorption with electrothermal atomisation is the most common technique used for the determination of total chromium in urine.[198,200] While direct analysis of biological samples is widely used in monitoring exposed workers it is unsatisfactory for the determination of trivalent or hexamlent chromium *per se*.[174,175]

True metal speciation must involve some sort of initial separation step, followed directly by analysis for each individual metal species present and separated.

Although a separate determination is possible employing the traditional spectrophotometric 3,5-diphenylcarbazide method, this

procedure is subject to interference from other ions and requires mineralization of the sample.[23] Slavin[253] reported that only Cr(VI) is chelated in ammonium tetramethylene dithiocarbamate (ammonium pyrrolidine dithiocarbamate) (APDC) and extracted in isobutyl methylketone (IBMK). Minoia *et al.*[174] reported the determination of Cr(VI) in urine samples after chelation with APDC, extraction with IBMK and atomic absorption measurement using a graphite furnace. The ETA-AAS determination of Cr(VI) in urine by solvent extraction separation with liquid anion exchanges (high relative molecular mass amines such as Amberlite LA-1 or LA-2) achieving a detection limit of 0.1 μg/l has been recently reported.[175]

Trace analysis and speciation for Cr(VI) and Cr(III) in various water and biological samples via HPLC-direct current plasma emission spectroscopy (HPLC-DCP) has been recently described by Krull *et al.*[145] Cr(III) and Cr(VI) ions are soluble in aqueous media compatible with HPLC injection/separation methods. The HPLC separations of the two chromium ions was readily accomplished using paired-ion, reversed phase conditions with either a tetrabutyl ammonium counter-ion or a camphor sulfonate counter-ion in solution. The order of elution of the two Cr ions was completely reversed in going from one counter-ion to the other in the mobile phase. HPLC-DCP technique permitted the detection for both Cr(VI) and Cr(III) in the range of 5 to 10 ppb, with at least 3 to 4 orders of magnitude linearity in the calibration plots. This was demonstrated with both retained and unretained HPLC conditions for both Cr species.

d) Selenium

There is increasing recognition that selenium is an important metalloid with environmental, biological and toxicological significance. It is an essential element in many species including human with the deficiency of this element in animal diets leading to serious diseases and its diminished levels in humans linked to several human cancers.[243] Although the role of Se in mammalian systems, in the enzyme glutathione peroxidase has been fully documented, the chemical form in which it occurs in selenoprotein is unknown.[81,82]

Selenium has four formal oxidation states: $-$II, 0, IV and VI. In natural waters, the principal dissolved species are IV and VI which

exist as selenite and selenate. Within particulate material, any of selenium's four oxidation states may be found. Since the difference between the essential and toxic concentration level of the element is rather narrow and the biological uptake and toxicity of selenium are controlled by its chemical form,[80,284,310] precise knowledge of the total selenium content as well as its valence forms in the environment, food products and biological tissues is mandatory.[2,53,57,116,142,208,228,232,243,251,294]

Lauwerys[150] has reviewed aspects of biological monitoring of Se in industrial exposures. Selenium concentrations in blood and urine appear to reflect mainly recent exposure. The normal concentrations in blood and urine are known to vary considerably depending on the nature of the dietary intake. The concentrations of Se in serum or plasma have been reported to range from 5 to 18 μg/100 ml.[296] The mean urinary concentrations of Se found in non-occupationally exposed persons by Glover,[96] Young and Christian,[315] Valentine *et al.*,[288] and Lauwerys[150] were 34, 7, 79 and 25 μg/l respectively. Although a biological threshold limit value for Se in urine of 100 μg/l has been proposed, it was noted that the epidemiological data supporting this proposal are very limited.[150]

The method generally employed for analyzing Se in biological materials initially involves the destruction of organic constituents with concurrent oxidation of the element to the tetravalent or hexavalent state and its subsequent determination by a variety of techniques. The most commonly employed techniques are AAS, fluorimetry, spectrophotometry, neutron-activation analysis, gas chromatography, and to a lesser extent X-ray fluorescence and cathodic stripping voltammetry.

The most widely used digestion methods for the determination of Se involves decomposition with acid mixtures such as $HClO_4$ and HNO_3,[14,49,209,211,224,266] HNO_3 and H_2SO_4,[46,161] HNO_3 and H_2O_2,[125] and H_2SO_4 and $HClO_4$.[118–120] Nygaard and Lowry have indicated that digestion mixtures such as HNO_3 and H_2SO_4, H_2SO_4 and H_2SO_4 and H_2O_2 and HNO_3 gave low recoveries of Se for organo compounds but found that the same digestion mixtures gave $\geqq 95\%$ recovery of the element when present in the inorganic form in aqueous solution. Several other workers[14,98,167,266] reported that wet digestion methods employing various acid mixtures yield up to 100% recovery of Se in biological materials.

A critical evaluation of some wet digestion methods for the cathodic stripping voltammetric determination of Se in biological materials was recently described by Adeloju *et al.*[3] A modified digestion procedure using a HNO_3 and H_2SO_4 mixture enabled adequate digestion of the sample material and retention of Se in a state amenable for determination and is suitable for cathodic stripping voltammetric determinations of Se in most samples (bovine liver, animal muscle, oyster tissue).

In general, total selenium contents have been determined after digestion of the matrix and separation of selenium by procedures including: hydride evolution,[105] by extraction of trimethylsilyl-derivatives,[36] by complexation with dithiocarbamate,[231] diamines such as 2,3-diaminonaphthalene (DAN),[105,142] or O-phenylene-diamine.[178] Employing substituted aromatic amines selenium could be detected with great sensitivity by electron capture detection in biological materials;[40,233,248,262] often with detection limits in the pg/ml range.[170]

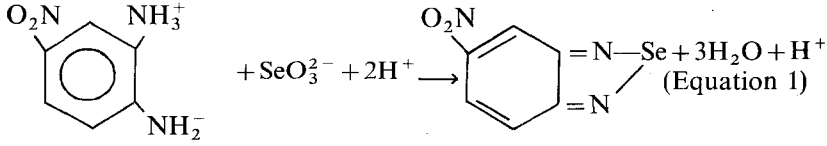
ETA-AAS is generally considered the most appropriate method to determine Se with high sensitivity and to cope with large sample batches encountered in routine analysis.[228] Normally the sample to be analyzed is at least decomposed in suitable acid mixtures as noted earlier (e.g., HNO_3, H_2SO_4, HCl and $HClO_4$). However, these conventional acids give rise to signal depression.[128,206] Because of interferences from a multiple matrix source, ETA-AAS is often combined with a separation method, e.g., solvent extraction,[180,189,206,235,265] ion-exchange,[210] ion-exchange and extraction,[121] volatilization of SeO_2 from organic materials,[172] or generation of SeH_2 and its collection in a cold trap.[138,217]

Gas chromatography in conjunction with various detectors is very suitable both for the determination of total levels of Se and for speciation.[228] The detection systems employed include: flame photometry,[109] thermal conductivity,[5] flame ionization;[231,239,299] electron capture,[127,223] emission spectrometry with microwave induced plasma excitation,[276] atomic absorption spectrometry,[45,105,126] and mass spectrometry[36] including separation step via stable isotope dilution-GC.[229]

Electron-capture gas chromatography, in particular, has evolved as a more sensitive and reliable analytical method for the determination of selenium. The procedure is based on the reaction of

tetravalent Se and various aromatic *o*-diamines to form piazselenols which can be extracted into organic solvents and measured by GC with detection by electron capture or microwave emission spectrometry. The *o*-diamines which have been used include: 2,3-diaminonaphthalene (DAN),[142,254] 3,5-dibromo derivatives[249] of 1,2-diaminobenzene. The above methods are specific for tetravalent selenium (selenite) and only the total Se content is measured. Hexavalent selenium (selenate) is also present in biological materials, but relatively few methods for its determination have been published. These include: spectrophotometry,[64] fluorimetry, and gas chromotography.[247]

Cappon and Smith[44] described a simple method for determining specific forms of selenium in biological samples. The procedure is based on the selective chelation of Se(IV) with 4-nitro-*o*-phenylenediamine to form 5-nitropiazselenol (Eq. 1) which is measured by electron-capture GC. Organoselenium and selenite (Se IV) are determined by digesting the sample in concentrated nitric acid and the total Se is determined by further treatment of the digest with hydrochloric acid. The difference between the two values obtained represents the selenate content. Selenium recovery ranges from 75–90% and was assessed by using ^{75}Se-labeled traces for liquid scintillation spectrometric assay. Gas chromatographic conditions allow the detection of Se concentrations below 1 ppb and mean deviation and relative accuracy averaged 2.3 and 3.4%. This method has been used in human population studies to assess Se–Hg concentrations and to examine the Se content and form in specific protein fractions of fish muscle. The formation of 5-nitropiazselenol from Se and 4-nitro-*o*-phenylenediamine is as follows:



(Equation 1)

4-nitro-*o*-phenylene diamine 5-nitropiazselenol

Koh and Benson[142] reported a critical re-appraisal of fluorometric determination of Se in biological materials. The reaction of 2,3-

diaminonaphthalene (DAN) with Se(IV) to form a fluorescent method for determination of Se. With the aid of metabolically incorporated ^{75}Se the method was critically re-examined. The study of Koh and Benson[142] showed that loss of ^{75}Se was negligible when liver or blood was microwave-dried or thermally dried at temperatures up to 120°C. During HCl reduction of Se(VI) to Se(IV), a temperature up to 210°C could be used with no loss of ^{75}Se. It was also found unnecessary to perform pH adjustment of solution for formation of Se-DAN complex before solvent extraction and this complex could be left in contact with aqueous phase up to one week under fluorescent light with no effect on analytical results. An improved single tube method was developed and within-batch variation of the improved method was about 2%, while the between-batch variation over a period of two years was less than 10%. The method of Koh and Benson[142] can handle 200 samples per batch and is applicable to a wide range of biological samples including liver, blood, urine, milk, hair, fish.

In liver and other animal tissues, inorganic forms of Se such as sodium selenite (IV) can be reduced to hydrogen selenide (−2 oxidation state) (at physiological pH values, H_2Se exists mainly in the form HSe^-) through a combination of nonenzymatic and enzymatic reactions involving glutathione and glutathione reductase.[91] It should be noted that H_2Se may be an important intermediate in the utilization of Se for the detoxification of selenium.[91] Ganther and Kraus[92] recently described the identification of hydrogen selenide and other volatile selenols by derivation with 1-fluoro-2,4-dinitrobenzene. The selenols react to form stable dinitrophenyl (DNP) selenoethers that can be extracted into benzene and are easily identified by HPLC or mass spectrometry. Hydrogen selenide is trapped in 90–99% yield primarily as the di-DNP-monoselenide with a trace of di-DNP-selenide.

The metabolism of selenium in living organisms is undoubtedly quite complex and the form which occurs within the living system depends on the form supplied.[90] The possible metabolic interrelationship between organic and inorganic forms of selenium have been described.[270] The biological mechanisms involve reduction and methylation and while little is known about the pathways by which the different forms of Se are metabolized to trimethylselenonium (TMSe) they are generally believed to involve a detoxification

mechanism.[84,184] TMSe is an important urinary metabolite at doses of selenite believed to be insufficient to trigger the respiratory excretion of dimethylselenide.[27] Blotcky *et al.*[27] recently reported the determination of trimethyl selenonium ion in urine by ion-exchange chromatography selectively capturing the TMSe on the cation exchange resin with recovery of $91.9 \pm 7.6\%$ with TMSe fraction subsequently irradiated with neutrons and radioassayed for ^{77m}Se activity.

A comparison of some determination procedures for selenium is illustrated in Table X.[228]

e) Arsenic

Human exposure to inorganic arsenic compounds occurs mainly via inhalation of industrial dust and via ingestion of contaminated food or drinking water. Exposure to organic arsenicals occurs mainly through compounds biosynthesized across the food chain. These compounds are characterized by a high absorption rate and exhibit a metabolic behavior and degree of toxicity different from that of inorganic arsenic. Hence the qualitative and quantitative determination of specific chemical species in addition to total inorganic element analysis is of particular importance for arsenic compounds whose environmental and mammalian toxicological hazards vary widely with molecular form.

Information on the behavior of arsenic in the human body has often been conflicting in regard to the accumulation of As^{+3} in the body,[158] whether As^{+3} is oxidized to As^{+5} and whether inorganic arsenic can be methylated in the body.[30,55,85] The current consensus is that biotransformation occurs in the body from trivalent to pentavalent form and from inorganic to methylated forms which may serve as a detoxifying mechanism[85] by a mechanism and at a site not definitively known.[162] Exposure to arsenic or its derivatives and ingestion or absorption by the skin and respiratory tract results in the major portion of arsenic being excreted in the urine, a small portion in the faeces and through the skin, hair and nails and possibly a trace through the lungs.[306]

Pentavalent and trivalent inorganic arsenic and methylated species, monomethylarsonic acid (MMAA; $CH_3AsO(OH)_2$) and dimethylarsinic acid (Cacodylic acid) [DMAA; $(CH_3)_2AsO(OH)$]

TABLE X

Comparison of some determination procedures for selenium (Raptis *et al.*, 1983).

Method	Matrix	Decomposition/ pretreatment	Separation	Detection limit	References
ETA-AAS	Blood plasma, serum	Dilution with HNO_3	—	9.0 ng/ml	Kumpulainen[147]
ETA-AAS	Blood	Matrix modification	—	0.25 ng	Tada *et al.*[275]
X-RFA	Biolog. material	Acid digestion	Precipitation	140.0 ng/g	Reptis *et al.*[228]
PIXE	Blood	Dry ashing	—	10.0 ng/ml	Berti *et al.*[22]
INAA	Biolog. material	—	—	20.0 ng/g	Gladney *et al.*[94]
DPCSV	Biolog. material	Decomposition sol. dilut. with buffer	—	0.16 g/ml	Paul and Daniel[214]
ASV (RGO ELECTR)	Biolog. material	Acid digestion	—	0.04 ng/ml	Andrews and Johnson[12]

have been found in the urine of both non-exposed humans,[13,30,83] and those occupationally exposed to inorganic compounds of arsenic.[33,83,257,307] After administration of small quantities (<1 mg) of inorganic forms of arsenic (as As(V)), e.g., as arsenate (H_2AsO_4, $HAsO_4^{2-}$, AsO_4^{3-}, As(III)), e.g., as arsenite ($H_2AsO_3^-$, $HAsO_3^{2-}$, AsO_2^-) to human volunteer subjects and in animal experiments, the urinary elimination of As in methylated form, principally as DMAA has been observed in humans,[33,34,55,162,205,279,313] as well as in a number of mammalian species.[216,285,287]

Several factors influence the route of arsenic excretion including As valence state. Pentavalent arsenic or arsenate is rapidly excreted whereas trivalent arsenic (as arsenite) tends to accumulate in the body according to Schroveder and Balassa.[238]

The determination of arsenic and its speciation in the occupational and the general environment as well as in biological samples is thus of critical importance and justifies the continued development of improved techniques for the speciation of these samples.

Although the literature regarding the determination of arsenic in biological material is abundant, it is nevertheless at times conflicting and confusing mainly because As may exist in different chemical forms *in vivo*. The forms studied most frequently are inorganic trivalent and pentavalent arsenic with their well-recognized different toxicities, their mono- and dimethyl derivatives as noted above represent the main metabolites *in vivo*.

Lauwerys[150] reviewed aspects of biological monitoring for industrial exposures to arsenic and Lauwerys *et al.*[151] and Buchet *et al.*[33] recently reviewed the techniques proposed for measuring the concentration of total As and that of some specific arsenic species (As^{+5}, As^{+3}, aromatic and alphatic arsenic derivatives) in biological materials, mainly in urine.

Until the early 1970s the analysis of arsenic generally involved digestion and determination of the total arsenic concentration as arsine, AsH_3, without regard to its origin. The most often used method is volatile hydride generation with $NaBH_4$ followed by various separation and detection systems. Separation of the volatile arsines is achieved by chromatography[11,278] or sequential volatilization.[56] The detection systems utilized have included: microwave emission spectrometry,[278] electron capture and flame ionization detection,[11] discharge emission, and atomic absorption spectrometry.[11,242]

A method to distinguish As(III), As(V) and organo-arsenic compounds was developed by Yasui *et al.*[314] involving extraction of As(III), reduction of As(V) to As(III) and re-extraction of As followed by digestion and generation of arsines with atomic absorption detection. Howard (1981) varied the pH of a solution to control selective reduction and sequential evolution of arsines, from As(III), As(V), monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA) employing atomic absorption. The procedure by Talmi and Bostick[277,278] for the determination of As(III), As(V), MMAA, DMAA and several alkyl arsines involved digestion and conversion of arsenic compounds to the corresponding arsines followed by either cold trapping or extraction into an organic solvent. Subsequent analysis employed a microwave induced plasma (MIP) for specific element detection.

The combined method of gas chromatography with a multiple ion detection (GC-MID) system and hydridegeneration-heptane cold trap (HG-HCT) technique for the quantitative determination of inorganic As and monomethyldimethyl-, and trimethylarsenic compounds was reported by Odanaka *et al.*[204] Arsine and methylarsines produced by $NaBH_4$ reduction are collected in *n*-heptane ($-80°C$) and then determined by GC-MID. The limit of detection for a 50-ml sample was 0.2–0.4 nanogram/ml of As for arsenic compounds.

A modification of (AG-AAS) of the arsine selective volatilization technique of Braman and Forebach[30] was developed by Buchet *et al.*[33,34] allowing the use of AAS detection instead of the plasma excitation emission spectrometer used by the authors. This method compared to the classical ETA-AAS, proceeded by dry ashing of the sample with MgO and proved to be quite satisfactory when urine from subjects who had not eaten fish were compared. The determination of urinary As by the procedure of Buchet *et al.*[33,34] appears to be a most suitable method for the biological monitoring of workers exposed to inorganic As. It is not influenced by the eventual excretion of As from seafood origin. Additionally, the relative proportion of the inorganic form of As versus the dimethylated form could yield information concerning the length of time between exposure and urine sampling.[33]

Lauwerys *et al.*[151] note that whether the relative concentrations of the inorganic, aliphatic and aromatic forms of arsenic found in human urine represent their true distribution in the urine secreted by

the kidney remains to be further investigated since possible changes can occur in the valence state in the bladder mainly at alkaline pH as well as breakdown of arsenical derivatives during urine pretreatment. It was also noted that no available system has been found that will digest tissue without changing the original valence state of arsenic, hence only total arsenic can be measured with accuracy in tissues.[33,151]

With the exception of neutron activation analysis, the usual techniques available (e.g., spectrophotometry, atomic absorption, polarography, gas chromatography) for determining total arsenic concentration in biological materials require a pretreatment of the samples. Additionally, it is noted that different samples pretreatments permit the specific determination of some arsenic derivatives.[151]

Although neutron activation analysis is recognized to be one of the most sensitive methods for the determination of total arsenic concentration in biological samples with a detection limit of 0.1 ng using a thermal neutron flux of 10^{-12} neutrons/cm^2 sec the cost of the analysis and access to a reactor limits its utility. In the procedure of Buchet *et al.*[33,34] for measuring total arsenic concentration in urine, the digest is acidified with a mixture of concentrated $HCl/HBr/HClO_4$ (10:3:3 by volume) and trivalent arsenic is extracted with toluene in the presence of KI. A back-extraction of the organic phase with an aqueous solution of cobalt nitrate yields an As-Co complex which can be measured by flameless atomic absorption spectrophotometry. The absolute limit of detection is 0.1 ng and thus by using 5 ml of urine the relative limit of detection of the technique is 2 μg/l.

The biological monitoring of workers chronically exposed to inorganic arsenic in industry has frequently been carried out by measuring the total amount of arsenic present in urine collected at the end of the shift or at the beginning of the next shift.[219] It should be noted that when the workers have not been instructed to refrain from eating fish or shellfish for 2 to 3 days before urine collection misleading results can be obtained by measuring total arsenic. According to Lauwerys,[150] the determination of inorganic arsenic, monomethyl arsonic acid and dimethylarsinic acid (cacodylic acid) in urine appears to be the method of choice for the biological monitoring of workers exposed to inorganic arsenic since these determinations are not influenced by the presence of organoarsenicals from

marine origin. It has been estimated by Buchet *et al.*[34] that a time-weighted average exposure to 50 μg inorganic As/m^3 would lead to an average urinary excretion of 220 μg As (sum of inorganic arsenic, monomethyl arsonic acid and cacodylic acid) per gram creatinine (urine collected at the end of the shift after a few days of exposure). In contrast, in non-occupationally exposed individuals exposed to arsenic, the sum of these three arsenical species does not usually exceed 20 μg/g urinary creatinine,[150] although high values can be found in geographical areas where the drinking water contains significant amounts of arsenic.

A NIOSH recommended method (P&CAM140)[184] for determining arsenic in urine is an arsine generation-colorimetric technique employing silver diethyldithiocarbamate. The range of this method for 25 ml of urine is from 0.01 mg/l to 1.0 mg/l. An additional NIOSH procedure[135] for the determination of arsenic in urine involves anodic stripping voltammetry (ASV) with a composite gold graphic electrode. The principle of the method involves the initial wet-ashing of the urine with a mixture of nitric, perchloric and sulfuric acids. The resulting pentavalent arsenic is reduced to trivalent arsenic with an acidic solution of sodium bisulfite and ferrous perchlorate. An acid solution of hydrazine dihydrochloride is added to prevent air oxidation of Fe^{+2} ions and the chemically reduced sample then analyzed by anodic stripping voltammetry. The detection limit of the method is estimated to be about 16 ng/ml of arsenic in a 1.0 ml sample of urine. The linear range of the electrode response is estimated to extend from the detection limit to 600 ng of arsenic. This upper limit corresponds to 600 μg/l of arsenic in a 1.0 ml sample of urine. The precision of the method (CV_T) is 0.08 over the above range.[195]

An arsine evolution-electrothermal atomic absorption method for the determination of nanogram levels of total arsenic in urine and water was described by Cox (1980). The procedure involves wet digestion with nitric, sulfuric and perchloric acids to ensure decomposition of organo-arsenic compounds, especially dimethyl arsinic acid (cacodylic acid) which accounts for 50–65% of the arsenic in urine and is highly resistant to decomposition. Sodium borohydride, a redesigned generator and an electric-heated absorption tube, are used for arsine evolution (from As^{+3}) and its conversion to atomic arsenic. The method has a detection limit of 6/ng ml, a sensitivity of 1 mg/ml and is linear from 0 to 110 ng/ml of arsenic.

It has also been suggested that the determination of arsenic concentrations in workers' blood specimens may also serve as a monitor of arsenic exposure.[196] The arsenic concentration in blood (as in urine) reflects mainly recent exposure. The biological half-life of arsenic in blood is about 60 hours.[165] Lauwerys[150] has stated that "there is not sufficient information in the literature to establish the relationship between the intensity of arsenic exposure and its concentration in blood nor to judge the value of measuring the different arsenic metabolites in blood".

The NIOSH (P&CAM192) procedure for the determination of arsenic in blood is based on anodic stripping voltammetry (ASV) with a composite gold-graphite electrode (CGGE). The detection limit was estimated to be about 16 ng/ml in a 1 ml sample of blood and the linear range extends from the detection limit to 600 ng of arsenic (0.016 to 0.6 μg/ml) in 1 ml of blood. The range can be extended by analyzing a smaller blood sample or by diluting the sample digestate with the acid solution of hydrazine dihydrochloride and taking an aliquot of the diluted solution for the ASV determination.

Davis *et al.*[60a] described the analysis of total arsenic in urine and blood at the ppb level by high-speed anodic stripping voltammetry. The method requires only 2 ml of blood and 1 ml urine. The samples were wet ashed with a mixture of nitric, perchloric and sulfuric acids and the ashed material was subjected to a procedure involving reduction and distillation to reduce AS^{+5} to AS^{+3} and to separate arsenic from the sample matrix. Collected arsenic was then quantitated by anodic stripping voltammetry (ASV) at a gold film electrode. The ASV analysis time was only 2 minutes. The method was as accurate, precise and reliable at the nanogram level as the more universally accepted colorimetry techniques are at the microgram and milligram levels. Method precision ranged from ± 1.4 ppb at the 5 ppb level to ± 0.96 ppb at the 25 ppb level and accuracy was estimated at $\pm 6\%$ over the range 5 to 500 ppb arsenic.

Arsenic determination in hair is generally considered to be unreliable for monitoring workers' exposure since it is difficult to distinguish between externally deposited arsenic and that systematically incorporated into hair.[33,312] However, the determination of arsenic in hair may be more useful for evaluating the environmental exposure of the general population to inorganic arsenic. The arsenic

levels in hair of non-occupationally exposed adults are usually below 2 mg/kg.[289]

The speciation of the chemical forms of As in the biological monitoring of exposure to inorganic As was recently reported by Foa *et al.*[83] and Buratti *et al.*[37] Total As content was determined in blood and urine by means of an AAS method that involves reduction of As to its volatile hydride and ashing at 600°C with MgO and $Mg(NO_3)_2$. Separation of inorganic As, monomethyl arsonic acid (MMAA) and dimethylarsinic acid (DMAA) by ion-exchange chromatography (on AG50W-X8 resin) followed by direct AAS allowed the determination of each As species in the urine. In a reference population of 148 subjects with only normal environmental exposure to As, total As concentration in the urine averages 17.2 ± 11.1 μg/l. Urinary As consists of 10% each of inorganic As, MMAA and DMAA, the remaining 70% consisting of other forms of organic As. Blood As concentration averages 5.1 ± 6.9 μg/l and correlates significantly with the urinary concentration of inorganic As and the sum of its metabolites (inorganic As, MMAA and DMAA).

In workers exposed to As_2O_3, inorganic As, MMAA and DMAA are the only chemical forms of As excreted in the urine that are relevant to a study of occupational exposure. Blood As concentration is proportional to exposure and correlates only with urinary DMAA excretion and hence DMAA seems to be the most appropriate single indicator of exposure according to Foa *et al.*[83] At high levels of exposure (total As excretion above 200 μg/l) As accumulates in the organism and DMAA excretion (total As excretion below 50 μg/l), a short-term accumulation does not occur, and the best biological indicator of exposure is organic As excretion. It should also be emphasized that seafood ingestion results in a marked increase in urinary excretion of total As that lasts for 24–48 hr and is not accompanied by any increase in organic As, MMAA or DMAA excretion. Since organic As from seafood does not mix with the pool of inorganic As in the organism it may be separately detected in the urine. Foa *et al.*[83] stressed that in the biological monitoring of human exposure to As, *particularly* in the case of high urinary values, the speciation of the chemical form of As in urine is vital, in order to establish as unambiguously as possible the source, industrial or alimentary, of exposure. Lowell and Farmer[162] described a

procedure for arsenic speciation in urine from humans intoxicated by inorganic arsenic compounds. Trends in the urinary concentrations of four As species, As(V), As(III), monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) were followed by several days subsequent to the acute intoxication of two human subjects by arsenic trioxide and sodium orthoarsenate ($Na_2HAsO_4.7H_2O$), respectively in unsuccessful suicide attempts. Total arsenic concentrations ranged from 1.6 to 18.7 mg/l. The increasing predominance of the less toxic methylated species, especially DMAA, after three or four days, supports the concept of methylation as a natural detoxification mechanism as part of an overall reduction/methylation sequence involved in the biotransformation of inorganic As by the human body. However, it was noted that there is an additional possibility of oxidation of As(III) to As(V) *in vivo* under extreme immediate post-ingestion conditions as suggested by initial high urinary As(V) after arsenic trioxide intoxication. The analytical procedure employed by Lovell and Farmer[162] was a modification of the method of Grabinski[99] for aqueous samples. The separation of As(III), MMAA, As(V) and DMAA from a 1 ml urine sample (diluted with 1 ml H_2O) was achieved on a combined anion/cation-exchange resin composed of Dowex AG50W-X8 and Dowex AG1-X1 with successive eluants of 0.065 M and 0.2 M trichloracetic acid. The eluant was collected for analysis by hydride generation-AAS.

The presence of dimethylarsinic acid (DMAA) in biological samples can cause an underestimation of total As content when analyzed relative to inorganic As standard by direct flame AAS. Webb and Carter[303] described a digestion procedure that quantitatively recovers DMAA as well as monoethylarsonic acid, inorganic As(III) and As(V) from aqueous and biological samples.

Methylated arsenicals were converted to inorganic As by wet digestion with HNO_3, H_2SO_2 and $K_2Cr_2O_7$ and subsequently to As(III) with sodium iodide. Arsenic was generated with $NaBH_4$ and converted to atomic As following immediate introduction into a nitrogen-generated air-hydrogen flame. This procedure produced a linear relationship to absorbance within a mass range of 50 to 300 μg As/arsine reaction. A sensitivity of 2 nanograms As and a detection limit of 7 nanograms arsenic/arsine reaction were also obtained. Recovery of DMAA from water, urine, faeces or whole blood ranged from 92 to 105% with a coefficient of variation of 5 to 10%.

The spectrum of As and Se via anion-exchange HPLC with sequential plasma emission detection has been described by McCarthy *et al.*[173] Detection limits were 1.8 mg As/sec and 3.9 ng Se/sec. Absolute detection limits were 52 ng As(III), 140 ng Se(IV), 57 ng As(V) and 91 ng Se(VI). Reproducibility at the 2.8 μg level was found to be 4%, 7%, 6% and 6% respectively for As(III), Se(IV), As(V) and Se(VI) with a linear response through 9 μg for the lower oxidation states and 5 μg for the higher oxidation states.

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