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High-performance, flow-based, sample pre-treatment and introduction procedures for analytical atomic spectrometry†

Plenary Lecture

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Recent and on-going work in the author’s laboratory is described with particular reference to the use of flow injection, continuous flow and HPLC procedures for the development of improved analytical methods. For (a) the determination of trace concentrations of As, Cd, Pb and Se and (b) the determination of various arsenic and selenium compounds. The methods have been applied to the analysis of several different sample matrices, including urine, soil, sediments, waters, plants (garlic, onion, apple leaves), yeast, fruit juices, wine, calcium supplements and marine plankton. The dependence of the LOD of an FI HG method on sample volume is examined and the validity of the proposed rectangular hyperbolic relationship established for a number of different analyses. The use of immobilized tetrahydroborate in conjunction with preconcentration of the analyte on the same anion-exchange resin is described as a possible procedure for improving the LOD. When used in conjunction with ETAAS, an LOD of 0.004 μg l⁻¹ for both As and Se was obtained for a sample volume of 10 ml. The procedure was also used in a method for the determination of inorganic arsenic and methylated arsenic(v) species. Methods for the determination of Pb by HG in the presence of hexacyanoferrate(m) were developed and applied to the analysis of urine, soils, waters and apple leaves. In the case of urine, the interference from the chelating agents used in the treatment of patients with elevated lead was overcome by the addition of S. The best LOD of 0.03 μg l⁻¹ was obtained for a procedure in which the lead hydride was trapped on the interior of a flame-heated slotted quartz tube under fuel-lean conditions with subsequent atomization when the flame was made fuel rich (by the injection of a small volume of isobutyl methyl ketone). It has been confirmed that it is possible to determine Cd by a ‘cold vapour’ procedure and it was shown that the nature of the atom cell surface played no part in the atomization process, which appeared to be the spontaneous decomposition of the species evolved from acid solution of cadmium on the addition of sodium tetrahydroborate solution. A modest increase in signal was obtained in the presence of nickel and thionine. An LOD of 0.02 μg l⁻¹ was obtained for a sample volume of 300 μl and the procedure was used for the analysis of NIST SRM 2711 (Montana Soil) in which the interference from the high lead content was overcome by coprecipitation with barium sulphate. Three examples of procedures using manifold designs incorporating an ‘eight-port’ rotary valve are given to illustrate the versatility of this component: the separation of high concentrations of uranium (5000 mg l⁻¹) from trace concentrations (1 μg l⁻¹) of Al, Be, Li and Mg for determination by ICP-MS, the automated implementation of the co-immobilization of analyte and tetrahydroborate on an anion-exchange resin and the stopped-flow microwave digestion of human urine for the determination of Se by HG and ETAAS. Improvements in the son-pair (with trichloroacetate-reversed-phase (C₈) HPLC procedure (with ICP-MS detection) for the separation of selenoamino acids (and closely related compounds) were made with a new staticky phase (Waters SymmetryShield RP₂) and a small-volume spray chamber. The results of extraction procedures indicated that much of the selenium in yeast and garlic is bound in high molecular mass material. So far only a few of the compounds in the extracts have been identified by retention time matching. A reversed phase (C₁₈), ion-pair (tetrabutylammonium) HPLC procedure for the separation of four immuno-reactive species (arsenate, arsenate, monomethylarsinate and dimethylarsinate), with detection by post-column HG-AAS, has been devised and applied to the extracts of soils spiked with these four species. Low recoveries of arsenite were obtained and microwave energy significantly accelerated the oxidation of arsenite to arsenate.

There are many areas of scientific study in which information about the chemical composition, in terms of the trace or minor elemental composition of the relevant materials, is needed as part of the problem-solving strategy. A good example of such an area is the study of the biogeochemical cycling of the elements. For some elements, only the most rudimentary knowledge has so far been accumulated, and for others, the relative magnitude of the contributing processes are unknown. There are many subsidiary problems to be tackled, such as the elucidation of the chemical basis of the cancer-suppressing and cancer-promoting properties of some elements and their relevant compounds.

In a recent overview of the future of atomic spectrometry for environmental analysis, Sturgeon‡ identified a number of trends and driving forces which probably have general applicability in the wider context of trace element determinations, namely: (a) more elements will be sought at lower concentrations, (b) total element determinations are becoming less relevant and speciation is becoming more relevant, (c) there is a greater need to minimize contamination as a consequence of enhanced instrumental LODs, and (d) there is a need for reduced waste production and reagent consumption. Sturgeon also pointed out that ‘Real sample matrices may


give rise to a plethora of potential problems, often associated with high dissolved solids content, including suppression of sensitivity, increased background levels and spectral interferences. The practical consequence is that, if the effects cannot be remedied by simple dilution, matrix separation and concentra-
tion of the analyte(s) becomes necessary. This is often the
case in any event, as many samples from uncontaminated sources contain analytes of interest at concentration levels which
challenge the best instrumental limits of detection. With
regard to the future, he suggested that ‘Automaton of many
routine sample preparation tasks can be anticipated. Enhanced
LODs can be achieved with direct solids (e.g., LA) and slurry
sampling approaches as well as through more extensive use of
on-line chemical manifolds and FI techniques to minimize
sample contamination and perform matrix separation–analyte
concentration functions. … Without doubt, the greatest impact
on sample processing and introduction for atomic spectrometry
has derived from the fields of FI technology and microwave
radiation’.

Finally, Sturgeon concluded, ‘Current instrumentation is
already capable of providing instrumental detection limits far
superior to method detection limits for many elements due to
our inability to control contamination and, hence, the method
blank. It is to be hoped that the widespread use of FI


techniques and, ultimately, perhaps nanotechnology for sample
handling will help alleviate this particular problem’.

However, if one takes a broad view of the current research
and development efforts into improving analytical method-
ologies in which commercially available atomic spectrometric
instrumentation is used, then it is apparent that the commonly
used techniques have limitations which, in turn, set perform-
ance limits to the methods in which they are incorporated.
Thus, FAAS does not have LODs low enough, it has a limited
working range and limited element coverage and suffers from
matrix interference; ETAAS suffers from the last three of
these limitations, but has instrumental LODs which are so low
that method LODs are governed by blank contamination; ICP-ES
has a wider elemental coverage (and offers the possibility of
genuine simultaneous multi-element determina-
tions), but has an upper range beyond which instrumental LODs
are not good enough for many environmental and clinical appli-
cations, and suffers from spectral interferences; and ICP MS
has very low instrumental LODs (blank contamination becomes
a problem again), with wide elemental coverage and nearly
simultaneous multi-element determinations but the instrumen-
tation is delicate and suffers from various matrix interference
effects. It is clear from the review literature covering these

techniques (as exemplified by the Atomic Spectrometry
Updates which appear in the June, August and October issues
of this journal) that much of the published work relating to
the use of these techniques concerns some aspect of the
limitations listed above. For some techniques, e.g., ETAAS,
the majority of technique-based publications are concerned
with achieving a separation between analyte and matrix (either
by the judicious choice of some thermochemical reactions
within the atomizer, or by the use of powerful background


correction procedures, or both.

In essence, the contribution of FI techniques to this area of
analytical instrumental analysis has been to open up a number of
possibilities for the separation of analyte and matrix with
analyte concentration. As these two functions are almost
always achieved by appropriate chemical reactions, the use of
FI techniques in this fashion has been described1 as ‘putting
the chemistry back into analytical chemistry’.

Flow injection, analyte concentration and detection
limits

Numerous publications appear each year describing
procedures in which the LOD has been improved over that
which would be obtained by direct introduction of the sample
(as received or as produced by some dissolution and/or diges-
tion procedure). This is particularly so for methods in which
the authors wish to use FAAS as the instrumental technique.2
This goal is, in itself, reasonable; FAAS is, after all, a well
understood technique, is robust and relatively inexpensive,
and requires a minimum of operator skill. By far the most
popular procedure for analyte concentration and matrix
separation is solid phase extraction (SPE). In general, the
analyte from a relatively large volume of solution is selectively
retained by a solid reagent phase (by a variety of mechanisms)
and then released into a relatively small volume of eluent.

In addition, chemical vapour generation (CVG), i.e., HG
and CV procedures, are the procedures of choice3 for the
determination of As, Se and Hg, and probably also for the
determination of Sb, Bi and Ge. In general, the analyte is
selectively converted into a volatile derivative which is then
blown out of solution. Both of these procedures give rise to
an increased signal owing to the greater analyte mass flux
introduced to the atomizer than would be the case for the
conventional nebulization of the original sample solution.

When used for ETAAS (with in-atomizer trapping in the case
of CVGs), the procedures have the effect of introducing a
greater mass of analyte into the atomizer than that which
would have been introduced in a conventional sample volume
(say 20 μl). Again, an increased signal is obtained. It is
therefore tempting to argue, as many workers do, that this
increased sensitivity gives rise to increased detection limits
and that there is a relationship between LOD and sample volume
such that the LOD can be decreased to any desired value; it is
simply a matter of preconcentrating a sufficiently large
sample volume.

Detection limits and sample volume

A recent survey4 (by no means exhaustive) of relevant papers
in the area of HG-ETAAS found as many as 15 papers in
which this kind of claim was made. This is not surprising as,
in general, it is true that LODs are low enough when sample
volumes are large and LODs can be decreased to lower val-
ues. However, it is decided misleading to give the
impression that there is no limit to how low the LOD can be.

The relationship between these two quantities needs to be
examined a little more closely and, in particular, the situation
that pertains when an FI procedure is used.

In a recent paper on the determination of Cd by CVG-
ETAAS with in-atomizer trapping, Geonaga Infante et al.
provided5 the following information: ‘the detection limit was
calculated to be 60 ng l−1 for 1.4 ml…of course, lower deter-
mination limits could be obtained if higher sample volumes were
preconcentrated…7 ml…resulted in a detection limit of
13 ng l−1. A scatter plot (see Fig. 1) of these data with LOD
(on the ordinate) against sample volume, V (on the abscissa)
would have two points. The relationship between LOD, Cb
(μg l−1), and V (ml) could be the straight line, Cb = −8.39 +
10−4 V +0.0718. However, this would predict that the LOD
was zero for a sample volume of about 8.6 ml and that for
sample volumes greater than this, the LOD would be negative!
A more thoughtful analysis of the situation would lead to the
conclusion that the relationship would be more properly
described by one of inverse proportion, i.e., as the signal
(for a given concentration) is directly proportional to sample
volume, it might be expected that the LOD would be inversely
proportional to V. For the data in Fig 1, the relationship
would be Cb=b/V, where b is a parameter to be fitted. The
data, in fact, do not fit this function particularly well as the
two values calculated for b are 8.4×10−2 and
9.1×10−2 μg ml−2. Another iteration of the modelling process
is needed.

Detection limit is a function of signal-to-noise ratio and

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may be defined as the concentration giving a signal equal to three times the standard deviation of the blank signal. If the slope of the calibration is $S$ (in appropriate units) and standard deviation of blank signal is $s_b$, then the LOD is given by $C_{LOD} = 3s_b/S$. In general, it might be expected that both $S$ and $s_b$ would be functions of $V$, with $S$ directly proportional to $V$ for those situations in which the instrument response is directly proportional to analyte mass. In general, $s_b$ will be made up of a contribution from the instrument, $s_i$, and a contribution due to the response to the reagents (including the analyte contamination in the reagents), $s_r$.

For a batch procedure, the amounts of reagents are independent of sample volume, hence the LOD–$V$ relationship is $C_{LOD} = 3s_b/kV$, where $k$ is the constant of proportionality between sensitivity and sample volume.

The detection limit can be as low as one wishes, simply by increasing the sample volume, but there is a practical limit set by the amount of sample needed and the time taken to process this sample volume. For example, in the determination of Cd cited above, if this model were valid it would require a sample volume of about 88 ml to obtain an LOD of 0.001 μg l$^{-1}$, at a flow rate of 2.8 ml min$^{-1}$, it would take about 30 min to process just one sample. As sample processing times become this long, instrumental drift becomes a contributor to the noise.

In a flow-based procedure, whether it involves HG or SPE, the amount of reagent used is directly related to sample volume and thus the contribution to $s_b$ from $s_r$ increases in direct proportion to the sample volume so that $s_b = s_i + kV$ (where $k$ is the constant of proportionality relating $s_i$ and $V$) and $C_{LOD} = 3s_b/kV = 3(s_i + kV)/kV = 3s_i/kV + 3k$. This equation has the general form $C_{LOD} = b/kV + a$, and will be referred to as the flow injection detection limit (or FIDL) equation. For the data used as example the parameters $a$ and $b$ may be calculated (as two points are available) to give an FIDL equation $C_{LOD} = 8.23 × 10^{-3}/V + 0.00125$. This equation is also shown in Fig. 1.

### Detection limits, sample volume and reagent blank

A more detailed treatment of the problem, which attempted to identify the factors contributing to the $a$ and $b$ terms, has been presented, together with results for the determination of As by FI-HG-ETAAS for 10 sample volumes covering the range 0.156–1.56 ml. The data were consistent with an FIDL equation $C_{LOD} = 0.041/V + 0.007$, from which it was concluded, with a certain degree of subjectivity based on estimations of the confidence intervals about the LODs, that no significant improvement in LOD would be obtained for a sample volumes in excess of 1.00 ml (LOD 0.05 μg l$^{-1}$). The model indicated that the major contribution to the $a$ term was the concentration of analyte in the reagents. Results supporting this finding had already been obtained for the determination of Se in urine by FI-HG-ETAAS. It was found that the major source of analyte contamination was the borohydride reagent and (b) the best LOD (0.006 μg l$^{-1}$ in the solution introduced into the manifold) was obtained for an injection volume of 1 ml. However, more data are needed before it may be concluded that for all FI-HG-ETAAS procedures the optimum sample volume is 1 ml.

### Immobilized tetrabhydroborate reagent

It may be concluded that a significant improvement in LOD would be obtained for an HG procedure if the reagent volume were independent of sample volume. One possibility for HG procedures would be to use an immobilized tetrabhydroborate reagent. Tsilafladit and Fergur described such a procedure for the determination of As$^{III}$ and Se$^{IV}$ in which the tetrabhydroborate was immobilized on an anion-exchange resin and the hydride was generated on passage of an acidified sample solution. It was observed that the analyte could also be retained on the resin and, as the first stage in developing methods based on immobilized tetrabhydroborate reagent, the performance of a procedure in which analyte (in this case selenium) and the borohydride were co-immobilized was evaluated. It was found that (a) the optimum tetrabhydroborate concentration was 0.05%, considerably lower than values typically used in conventional flow or batch procedures, (b) the procedure gave signals for both As$^{III}$ and As$^{V}$ but only for Se$^{IV}$ (not for Se$^{VI}$) and (c) the LOD decreased as the sample volume increased. The procedure was applied to the determination of Se in river, lake and tap water; no interferences from these matrices were encountered for Se concentrations between 0.5 and 10 μg l$^{-1}$. Detection limits were measured for three sample volumes, 3, 6 and 9 ml, for which LODs of 0.15, 0.12 μg l$^{-1}$, respectively, were obtained. These results are consistent with the FIDL equation $C_{LOD} = 0.540/V + 0.006$ (the correlation coefficient of a least-squares fit of a straight line to points on a plot of $C_{LOD}$ versus $1/V$ is 1.0000).

### Analyte concentration by SPE

The next stages in the development of this procedure were (a) to separate the retention of the tetrabhydroborate and the analyte, so that the amount of reagent used was independent of the volume of sample loaded, (b) to trap the generated hydride on the interior of a graphite furnace and (c) to automate the procedure with a commercially available computer-controlled flow injection analyzer unit [a Perkin-Elmer (Norwalk, CT, USA) FIAS 200]. The separate sample loading step meant that the sample volume could be increased substantially without increasing the blank contribution and it was found that for a 20 ml sample volume (loaded at 20 ml min$^{-1}$), the LOD was 0.004 μg l$^{-1}$. The amount of tetrabhydroborate used per determination was 5 mg (5 ml of 0.1% m/v solution flowing at 5.0 ml min$^{-1}$ for 1.0 min). The LOD was significantly improved over that which would have been obtained for a sample volume of 20 ml with the quartz tube atomizer and the simultaneous immobilization procedure. Substitution in the above FIDL equation gives a value of 0.007 μg ml$^{-1}$. The procedure was applied to the determination of Se in several fresh garlic samples. Values ranging from 35 to 350 μg kg$^{-1}$ were found.

### Application to speciation of arsenic compounds

As the arsenic species As(OH)$_3$, $\text{As(O}_2\text{H})_3$, $\text{CH}_3\text{AsO}_2\text{OH}$, (MMA) and $\text{(CH}_3\text{)}_3\text{AsO}_2\text{OH}$ (DMA) are weak acids with sufficiently different $pK_a$ values, a procedure based on selective
analyze retention on the anion-exchange resin is possible. The pKₐ values are As(V), 2.3, As(III), 9.2, monomethylarsinate(V), 4.0 and dimethylarsinate(III), 6.3, hence at low pH values As(III) will exist as an anion whereas all the other species will be fully protonated and not retained. The results of the pH study are shown in Fig. 2, confirming that this predicted behaviour is indeed obtained. A speciation procedure was devised²⁴ based on selective retention of As(V) (at pH 2.3) and selective oxidation of the other species to As(III). Three stages were needed: in the first, only As(V) was retained; in the second, As(III) was selectively oxidized with hydrogen peroxide and nitric acid so that the difference between the result for this step and that for the first step gave the As(III) content; in the third stage, all species were oxidized to As(V) by alkaline peroxodisulfate in a sealed vessel in a microwave field, so the difference between the result for this step and that for the second step gave the concentration of the methylated arsenic species.

A fully automated system was constructed around a Perkin-Elmer FIAS 200 unit interfaced to an ETA atomic absorption spectrometer and the method applied to the determination of arsenic species in river and tap water. The sample was loaded at 10 ml min⁻¹ for 60 s, followed by 0.2% tetrahydroborate at 5 ml min⁻¹ for 45 s. The LOD for a 10 ml sample volume was 0.004 µg l⁻¹. This value may be compared (a) with the value of 0.04 µg l⁻¹ obtained for previous FI-HG-ETAAS determination of As for which a sample volume of 0.50 l was used and (b) with the predicted value from the relevant FIDL equation for a 10 ml sample of 0.011 µg l⁻¹, showing that a significant decrease in the contribution of the blank had been obtained by using the anion-exchange resin. A reference water sample (total As content 90 µg l⁻¹) was accurately analysed and the interferences of nine cations and eight anions on 0.30 µg l⁻¹ As were investigated.

**FI determination of lead**

Two approaches to the determination of lead have been under investigation. In the first, lead has been concentrated on a solid phase extractant and then eluted into a smaller volume of eluent for determination by FAAS. In the second approach, lead hydride has been generated, separated from solution and determined by AAS either with a quartz tube atomizer or, after in-atomizer trapping, with a graphite furnace atomizer. The possibility of in-atomizer trapping of lead in a slotted quartz tube in a flame has also been demonstrated.

**Analyte concentration by SPE**

Two manifold designs have been used for the concentration of lead as the diethylthiocarbaminate (DDC) complex by retention on a hydrophobic solid phase extractant material. In the first of these,²⁵ the lead DDC complex was formed in the FI manifold by merging the sample stream with a solution of DDC buffered at pH 4–5. The complex was retained on a small column of C₁₈ silica and then eluted with acetoniitrate. To avoid the loss of the DDC complex to the walls of the tubing, the confluence point was located inside the loop of the ‘injection’ valve so that in the ‘elute’ position all tubing in contact with the lead DDC complex was washed with eluent. This arrangement is shown in Fig. 3. The manifold was used in the determination of lead in water and fruit juice matrices at concentrations of 0.5 and 5 mg l⁻¹. The enrichment factor, calculated as the ratio of calibration slopes, was about 50 and any complex adhering to the walls of the tubing is also dissolved.

The lead–DDC complex was formed off-line at pH 9 by the addition of the minimum amount of a DDC solution to the sample. The lead–DDC complex was retained on the GC stationary phase Chromosorb 102 (a styrene–divinylbenzene copolymer) and eluted into a small volume of ethanol delivered from an injection valve. This manifold is shown in Fig. 4. An enrichment factor of about 25 was obtained with an LOD of 2 µg l⁻¹ for a sample volume of 4.4 ml (2 min loading). The method was applied to the analysis of spiked tap water and artificial sea-water at concentrations 0.1–0.4 mg l⁻¹. A soil SRM (NIST SRM 2711 Montana Soil, lead content 1162 mg kg⁻¹) was accurately analysed following digestion with nitric and hydrofluoric acid in a sealed vessel in a microwave field. No problems exist with sample loss to the walls of the tubing or with passage of sample matrix components to the nebulizer were encountered. This design of manifold would probably not be so suitable for use with ICP instrumentation which is less tolerant to the delivery of solutions of variable concentration.
Various conditions for the generation of plumbane have been investigated, resulting in the conclusion that the greatest sensitivity for FI-HG-AAS with quartz tube or graphite furnace atomization is obtained in the presence of hexacyanoferrate(III) as 'oxidant' and hydrochloric acid. These findings are in agreement with those of several other workers.

A method was developed for the determination of lead in human urine. After optimization of the various flow injection parameters, the LOD was 0.08 \( \mu g \) l\(^{-1} \) for a sample volume of 0.300 ml. A quartz tube atomizer, a Nafion dryer (between the gas-liquid separator and the atomizer) and peak height quantification were used. An RM [NIST SRM 2670 Trace Metals in Urine with Pb concentrations of 10 \( \mu g \) l\(^{-1} \) (normal) and 100 \( \mu g \) l\(^{-1} \) (elevated) was accurately analysed. However, when the possible interference of a chelating agent, such as EDTA (used in the treatment of patients with elevated lead), was investigated it was clear that the simple sample pre-treatment of addition of hexacyanoferrate(III) and dilution with hydrochloric acid solution would not be suitable for the analysis of 'real' urine samples.

The method was modified by the addition of Sc, whose EDTA complex formation constant is 10\(^5\) times larger than that for PbEDTA, and the lead signal was restored. The modified procedure was applied blind to the analysis of 50 samples provided by the New York State Department of Health. Samples were diluted between 5- and 100-fold, and the solution injected contained \( 2.5 \times 10^{-4} \) m Sc, 5% m/v hexacyanoferrate(III) and 0.1% HCl. Calibration with aqueous standards was possible. A comparison of the results of the determinations by FI-HG-AAS with those obtained by the New York State Department of Health (in whose laboratory an ETAAS procedure was used) is shown in Fig. 5, from which it can be seen that there is no significant difference at the 95% confidence level between these two sets of results.

The procedure has been further developed by trapping the hydride at 300 °C on the interior of a graphite furnace atomizer. The atomizer was first pretreated with Ir (120 \( \mu g \)) and the 95% confidence interval between these two sets of results.

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The procedure has been further developed by trapping the hydride at 300 °C on the interior of a graphite furnace atomizer. The atomizer was first pretreated with Ir (120 \( \mu g \)). The LOD was 0.12 \( \mu g \) l\(^{-1} \) for a sample volume of 1.00 ml. However, after purification of the hexacyanoferrate(III) by passing a solution through a cation-exchange resin, the LOD was decreased to 0.03 \( \mu g \) l\(^{-1} \). Unfortunately, this procedure did not prove viable for routine use as the capacity of the resin to retain lead rapidly became exhausted. The procedure was used in a method for the determination of Pb in calcium supplements. Two materials (CYS brand) were examined: one contained 333 mg Ca + 133 mg Mg + 5 mg Zn per tablet and the other contained 500 mg Ca (from crushed oyster shells) per tablet. In terms of the lead content, the former contained 0.57 mg kg\(^{-1}\) (i.e., 0.43 \( \mu g \) per tablet) and the latter 0.66 mg kg\(^{-1}\) (i.e., 0.43 \( \mu g \) per tablet). The method was vali-
dated by the analysis of the materials by ETAAS methods (three different sample pre-treatment procedures were used) and by spike recoveries. Further studies of the HG of lead are in progress as the LOD for ETAAS is no better than that for quartz tube atomization, although the sensitivity of the former is much higher. It is concluded that this noise associated with the ETAAS procedure is much higher, and as the only significant difference between the two procedures is the in-atomizer trapping stage, it is thought that this process is, for some reason as yet unknown, less reproducible for plumbane than for other hydrides such as arsine and hydrogen selenide. Clearly improved LODs would be obtained with purer hexacyanoferrate(II) and work is in progress to find a routine method for the removal of the lead from this reagent.

Finally, in this saga of method development based on plumbane generation, a procedure based on trapping the hydride on the interior of a slotted quartz tube atomizer has been devised.26 The concept of trapping atom precursors on a cool surface in a flame was first described by Lau et al.15 and the concept of increasing atom residence time in the flame by the insertion of a slotted quartz tube was first described by Watling.28 The combination of the two procedures was proposed by Ataman and co-workers,29,30 who showed that it was possible to trap the precursor species in a fuel-lean flame and atomize in a fuel-rich flame produced either by rapidly changing the fuel flow or by the pulse nebulization of an organic solvent. They also showed that it was possible to dispense with the water-cooled tube and trap on the slotted tube.25 This concept has been extended27 by the introduction of the lead as the hydride into the slotted quartz tube in a fuel-lean flame. Essentially the same generation chemistry was used as for the previously developed HG methods. The lead was atomized on the injection of 50 μl of suberyl methyl ketone. The LOD was measured for three different sample volumes, 2.6, 5.2, and 7.8 ml, for which the LODs were 0.075, 0.030, and 0.016 μg/kg respectively. This data, along with that for an FIDL plot of the equation $C_i = 0.175 V_i + 0.009$ with a correlation coefficient of 0.99. The procedure was used as part of a method for the determination of Pb in two SRMs: NIST SRM 2709 San Joaquin Soil (19 mg kg$^{-1}$) and SRM 1515 Apple Leaves (0.47 mg kg$^{-1}$) were analyzed accurately.

Work is in progress42 on the determination of lead by a combination of SPE (the DDC complex on Chromosorb 102) with HG from the column eluent. The PMDC was eluted with 0.3% ethanolic hydrochloric acid solution and merged with a hexacyanoferrate(III) stream prior to merging with tetrahydroborate. For quartz tube atomization, the LOD was 0.2 μg L$^{-1}$ (i.e., about a factor of 10 improvement over the value obtained for elution with ethanol and determination by FAAS). The procedure was used as part of a method for the determination of Pb in wine over the concentration range 5–25 μg L$^{-1}$. These results are interesting from two points of view: first, it appears that plumbane can be generated in the presence of DDC (the acid concentration may be high enough to release the lead from the complex) and ethanol, and second, it appears that DDC is capable of removing lead from the various complexing agents in the wine.

### Determination of cadmium

The SPE chemistry, based on the retention of the DDC complex on Chromosorb 102 with elution by acetonitrile, has also been used for the determination of Cd in waters and fruit juices.33 Detection limits were measured for three different sample volumes, 0.79, 2.36 and 7.08 ml, for which values of 1.3, 0.65 and 0.39 μg L$^{-1}$, respectively, were obtained. These data fit the FIDL equation $C_i = 0.799 V_i + 0.289$ with a correlation coefficient of 0.999.

### Chemical vapour generation

When tetrahydroborate is introduced into an acidified solution of cadmium, a volatile derivative is formed which can be either transported into a quartz tube atomizer in the light path of an atomic absorption spectrometer or trapped on the interior of a graphite furnace atomizer. The absorption signal produced from either atomizer is proportional to the cadmium concentration in the original solution and this phenomenon forms the basis of a viable method for analysis of cadmium. Sanz-Medel and co-workers34 made the interesting observation that, with the quartz tube atomizer, the procedure worked satisfactorily even when the tube was at room temperature. They provided convincing spectroscopic evidence33 that the absorbing species were indeed cadmium atoms and they proposed, therefore, that the procedure be called ‘cold vapour’ by analogy with the corresponding procedure for the determination of Hg. It has been postulated that the species evolved from solution is cadmium hydride (CdH$_2$), but that this is sufficiently unstable that a significant proportion has decomposed to cadmium atoms during the time taken to pass from the gas–liquid separator to the atomizer. As cadmium atoms are not thermodynamically stable at room temperature with respect to the bulk metal, the cadmium atoms formed in this fashion are presumably kinetically ‘stable’. These results have been confirmed by Guo and Guo35 and Kradtapt.36 The best conditions for the generation of the precursor of the atoms are the subject of some debate. It has been reported that a surfactant should be present,34(b) that a considerable signal enhancement occurs in the presence of nickel, cobalt and thiosulphate34(c) and that potassium tetrahydroborate gives a significantly enhanced signal compared with that obtained in the presence of sodium tetrahydroborate.34 All of these claims have been investigated in some detail37 for a typical two-line flow injection system (i.e., the acidified sample is injected into an acid carrier which merges with an alkaline tetrahydroborate stream; after passage through a reaction coil, argon is merged and the mixture flows through a stripping coil to release the lead from the complex). The findings are that a surfactant has no effect, nor has potassium tetrahydroborate. In the presence of 1% thiourea and 10 μg L$^{-1}$ Ni or Co, the peak height signal is enhanced by 10–20% but a black precipitate rapidly forms in the presence of Co. In addition, it has been found that the surface of the atom cell plays no role in the atomization process. A Pyrex glass cell of smaller diameter than the ‘standard’ quartz cell can be used to increase the peak height signal. However, when a Nation dryer was used to transport the volatile species to the atom cell, the signal was lost completely. A small open chamber, of volume about 5 ml, was used as a gas-liquid separator, which diverted only 8% of the cadmium to waste. It was observed that better signals were obtained if the acid concentration in the injected sample was lower than that in the carrier and that the length of tubing between the gas-liquid separator and the atom cell should be the minimum value possible. All of the evidence suggested that the signal characteristics were due to the kinetic features of an unstable analytic species. Interestingly, peak height was a more precise and robust quantitative parameter than peak area.

Procedures were developed for the accurate determination of Cd in NIST SRM 2711 Montana Soil at 41.7 mg kg$^{-1}$ and NIST SRM 1515 Apple Leaves at 0.013 mg kg$^{-1}$. The high lead content of Montana soil (1162 mg kg$^{-1}$) interfered and was removed by coprecipitation with barium sulfate. In presence of thiourea and Ni, the slopes of the standard additions calibration and that for aqueous standards were not significantly different. The LOD was 0.016 μg L$^{-1}$ (only one value for a sample volume of 0.300 ml has been obtained so far).
The eight-port flow injection valve

While a variety of FI apparatus has been used in the experiments described above, the automation of several procedures has been greatly facilitated by the use of a two-position ‘eight-port’ rotary valve. This designation is somewhat of a misnomer, as the device actually has 16 ports, eight on the stator and eight on the rotor. To illustrate the versatility of the device, three different uses are shown. First, the device has been used in a manifold for determinations of Se and As by HG-ETAAS in which the hydrides have been generated on the passage of acid through a column of anion-exchange resin on which the analyte and tetrahydroborate have been sequentially immobilized. The manifold and its operation are explained in Fig. 6. Second, a manifold for the separation of analyte from matrix by retention of the matrix on a solid phase extractant has been designed around this device. The manifold has been used for the separation of uranium (at concentrations up to 5000 mg l\(^{-1}\)) from solutions of light elements (Al, Be, Li and Mg), allowing their determination at concentrations down to 1 mg l\(^{-1}\) by ICP-MS. The manifold and its operation are shown in Fig. 3 in ref. 38. Third, the device has been used in a manifold for the on-line microwave-assisted digestion of selenium compounds in urine, allowing the determination of Se in this matrix by HG. It has been shown\(^8\) that it is possible to convert all Se compounds in human urine to selenite, by heating under reflux in the presence of bromine generated from the reaction of bromate with hydrobromic acid, but attempts to adapt this sample pretreatment to an on-line format had proved unsuccessful in terms of producing conditions which would convert trimethyl-selenonium (TMSe) (the Se species found in human urine most resistant to oxidative attack) into selenite.\(^{25}\) However, recently it has been found\(^{39}\) that a manifold design in which the sample zone is isolated in a closed loop in the microwave field produces conditions under which TMSe is converted into Se\(^{IV}\). The manifold design is shown in Fig. 7. Under the same operating conditions, Se\(^{VI}\), selenocystine, selenomethionine and selenoethionine were also quantitatively converted into Se\(^{IV}\).

Speciation of selenium and arsenic

There is considerable research activity directed towards the development of methods for the separation and quantification of the various inorganic and organometallic species of arsenic and selenium found in environmental and clinical samples. Numerous chromatographic (mainly HPLC) and non-chromatographic procedures have been published, many of which incorporate AAS or ICP-MS detection.\(^{30-34}\) However, it appears that (a) is it difficult to achieve efficient (>1000 plates) LC separation of inorganic and methylated arsenic species in short times (<5 min) and (b) many clinical and
biological materials contain numerous organoselenium compounds which are poorly resolved at short elution times or are unidentified, or both.

Selenium speciation

As part of a broad-based study of the biogeochemical transformations of selenium compounds and of the nature of selenium compounds in materials with anti-cancer properties, analytical methods are under development in which selenium compounds have been separated by HPLC and detected by IC-ICP-MS. As the bulk of the studies so far have been concerned with plant and yeast materials, the targets have been amino acids and closely related species. Recent efforts have been devoted to improving the interface between the chromatograph and the spectrometer and to improving the performance of the chromatographic separation. Initial studies were made of ion-exchange separations and reversed-phase separations of amino acid derivatives. However, an ion-pair reversed-phase procedure had superior performance in terms of (a) ranging from about 60% at 0.1 μg l⁻¹ to over 90% at 100 μg l⁻¹. Sensitivity is a function of species, with a 28% difference between the most sensitive and the least sensitive compounds and, in the determination of total Se, the sensitivity is a function of the acid concentration remaining after the digestion is complete. This problem has been noted by other workers and it may be overcome to a large extent by the addition of a mixture of water-soluble tertiary amines (known as CFA-C amines).

Further modifications of the conditions were made so that the composition of the mobile phase was 1% MeOH + 0.6% trifluoroacetic acid (TFA) + 2.5 mg l⁻¹ Gx (as internal standard) adjusted to pH 2 with ammonia solution and the stationary phase was Waters SymmetryShield RP C₁₂. The improvement in chromatographic performance is shown in Fig. 8 for four selenium standard compounds. The injection volume was 10 μl and the mobile phase flow rate was 1.0 ml min⁻¹. Also contributing to the improved resolution were the use of a Methanol nebulizer and a spray chamber of volume approximately 14 ml containing a fixed 1 cm diameter glass impact bead.

Three different extraction procedures have been used. The first of these was a simple hot water extract as it is known that in the case of yeasts and allium vegetables, anti-cancer action is obtained with such extracts. The second was a water extract in the presence of an enzyme (protease XIV) which reduces polypeptides to dipeptides and the third extraction was with hydrochloric acid (0.1 M) + ethanol (95%). In each case the extraction was carried out for several hours, and the extract was filtered through a 0.45 μm filter and a 100,000 molecular mass cut-off filter (to remove polymeric material, including excess enzyme reagent). Total Se in the original material and in the residues after extraction and filtration was determined by a procedure in which the material was digested with nitric acid in a sealed vessel in a microwave field. This procedure was validated by the analysis of various reference materials. Although these studies are far from complete, some general observations have been made: for most materials a greater proportion of the total Se was removed by the enzymatic extraction than by the other procedures, suggesting that most selenium is incorporated into larger molecular structures that are broken down by the enzyme. The species profile depends on the material (not all yeasts give the same profile, for example) and the profile also depends on the concentration of the selenium. At this stage it difficult to draw any conclusions about the relative amounts of various selenium compounds which occur at natural concentrations. It has also been observed that for several different materials (including yeast, garlic, plankton, Brazil nuts and bacteria), the maximum amount of selenium that the organism can tolerate is about 2000 mg kg⁻¹.

A number of potential problems for the analytical methodology have also been identified: recovery of the selenium species from the column is a function of concentration, ranging from about 60% at 0.1 μg l⁻¹ to over 90% at 100 μg l⁻¹. Sensitivity is a function of species, with a 28% difference between the most sensitive and the least sensitive compounds and, in the determination of total Se, the sensitivity is a function of the acid concentration remaining after the digestion is complete. This problem has been noted by other workers and it may be overcome to a large extent by the addition of a mixture of water-soluble tertiary amines (known as CFA-C amines).

Initial work on the identification of the selenium compounds has been based on retention time matching with standards. However, most of the compounds are still to be identified as no match with existing standards has been found. More recently, the approach of derivatizing with reagents designed to identify particular chemical functionalities has been attempted. These reagents have included hydrogen peroxide (as an oxidant), thiosulfate (as a reductant) and methanol or ethanol with sulfuric acid (as an esterifying agent). In this procedure, the chromatograms before and after reagent addition are compared to identify (a) which compounds may be oxidized or reduced (and whether the process is reversible) or (b) which compounds may be esterified. Future studies will involve the use of LC-MS (electrospray ionization with an ion trap spectrometer) for which initial studies on known selenium compounds indicate that the spectral interpretation may not be so complicated (as the presence of Se in a fragment ion is easily deduced from the characteristic isotope pattern), but that the sensitivity of this form of LC-MS is comparatively low.

Arsenic speciation

A common preservative for structural timber is chromated copper arsenate. The timber is treated under pressure with an aqueous solution of the oxides. As part of an on-going study into the fate of arsenic leached from pressure-treated timber into soils, a method for the determination of As⁴⁰, As³³ and the two methylated forms of arsenate (discussed earlier) in soils has been under development. The hypothesis to be examined is that the inorganic arsenic which gets into the soil is transformed to volatile methylated species by the action of soil micro-organisms. Initially, an anion-exchange HPLC separation was devised and it was shown, on the basis of ultrasound-assisted extraction with methanol + hydrochloric acid (50% + 10%), that an arsenic-containing reference material, NIST SRM 2704 Buffalo River Sediment, contained arsenite and dimethyl arsinate. Following the success of the ion-pair procedure for the separation of selenium amino acids, work has been directed to the development of a similar procedure for the separation of these four arsenic species. A
procedure had been devised based on the use of a Waters Resolve C18 column with 6 mm potassium hydrogen phosphate + 2.5 mM tetrabutylammonium hydroxide + 0.25% MeOH at pH 5.7 (adjusted with phosphoric acid) as the mobile phase. For detection by post-column HG-AAS with quartz tube atomization, LODs between 0.5 and 2 μg l−1 have been obtained for a 100 μl injection volume. The chromatographic profile is shown in Fig. 9.

The extraction of these arsenic species from soil is currently being studied. Obtaining quantitative recovery without transformation is proving to be difficult. A variety of extractants have been used, including tetrabutylammonium hydroxide and phosphoric acid (at a variety of pH values with potassium dihydrogenphosphate as buffering agent). The possible roles of ultrasound and microwave energies are also being studied. So far it may be concluded that it is possible to extract MMA and DMA, but AsV is strongly bound immediately on spiking into a soil matrix and, so far, it has not proved possible to recover all of this species. The application of microwave energy results in the conversion of some AsV into AsIII and some of the earlier good recoveries for AsV results in the conversion of some AsV into AsIII into AsIII and therefore are called into question. This is a difficult analytical problem and there is still a long way to go with this method development.

Conclusions

Flow-based procedures, in which sample pre-treatment chemistry is performed in narrow-bore conduits, have a number of characteristics which can lead to the development of improved analytical methods. In addition to providing a closed, contamination-free environment for the handling of samples, flow procedures allow for the automated or semi-automated implementation of analyte and matrix separations that would be tedious, time consuming and prone to contamination if performed in a batch mode. In addition, many flow procedures allow for simple direct coupling between the sample pre-treatment stages and the instrumental measurement stage of the overall method. Flow procedures are a good way to implement solid phase extraction and chemical vapour generation procedures, although the usual increase in reagent consumption as the sample volume increases produces a rectangular hyperbolic relationship (the so-called FIDL equation) between sample volume and LOD which is not asymptotic to the concentration axis. There are, therefore, limits to the extent to which LODs can be improved by increasing the sample volume. However, in the case of chemical vapour generation, the use of an immobilized reagent removed the dependence of reagent blank on sample volume and a way has been opened up for further improvements in detection limits. The dependence of LOD on sample volume is a useful performance characteristic and the relevant information should be included in publications.

There are distinct possibilities for the use of HG in methods for the determination of Pb and Cd, and such procedures could become as popular as those for the determination of As and Se. The generation of lead hydride is greatly facilitated by the presence of hexacyanoferrate(III). Cadmium may be determined by AAS at room temperature.

For the determination of various selenium and arsenic species in environmental and food samples, separation by reversed-phase ion-pair HPLC with element-specific detection seems to be a viable stage in the overall method, despite some of the difficulties encountered related to recovery from the column and the dependence of sensitivity on chemical factors. In the case of the selenium compounds, the identification of species responsible for large numbers of peaks in the chromatograms remains a major area of further work. For both As and Se compounds, the problems of extraction of the compounds from solid matrices also remain as a major area for further work.

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