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ELEMENT SPECIFIC DETECTION IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: A USEFUL TOOL IN ENVIRONMENTAL ANALYSIS[1]

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An overview is given on possibilities to use element specific detection for high performance liquid chromatography (HPLC) in order to determine concentrations of different species of trace element compounds in different matrices. Examples on the determination of various selenium and chromium species are presented.

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Introduction

The determination of concentrations of trace elements in various matrices has become one of the main topics of analytical chemistry nowadays. Demands arise mainly from environmental, industrial and medical chemistry where monitoring of trace elements is required in many cases to improve the quality of the environment, the health of man, or the quality of products used for specific purposes.

The determination of the *total concentration* of many trace elements has become more or less routine in many analytical laboratories. In case that the element of interest can occur in more than one form, such determinations are, of course, necessary since they serve as a basis for subsequent analyses, but they are insufficient in many respects. Thus, during the past few decades, *speciation* moved gradually into the center of analytical chemical interests, a term which is not unambiguously used in the literature [1]. Usually, speciation means the determination of different species, in which an element occurs in the sample under investigation; the term will be used in this meaning also throughout the text presented here.

But why is speciation so important? Supposed that an element exists in different compounds in a matrix, it is evident that the value of results, which we can obtain by the determination of the total concentration only is rather poor. By speciation, on the other hand, we are able to obtain additional, secondary and with respect to final evaluations much more important data than those accessible solely by investigations dealing only with total concentrations. Such additional information can include, particularly with respect to environmental and medical chemistry:

- chemical information,
- toxicological information,
- biochemical and biogeochemical information.

Chemical information means that we finally know about the binding form, about the chemical nature and the concentration of compound at which the trace element exists in the sample. These are directly obtainable data of enormous importance, since all other further conclusions can be based on them.

Toxicological information is essentially dependent on chemical information. In general, different binding forms of the same element exhibit different toxicities for living organisms and, thus, also for man. As an example, Table I summarizes the toxicities for man of some naturally occurring compounds of an element, which is in ordinary peoples's opinion typically poisonous, i.e., arsenic. Arsenic is known to be present in various compounds in the environment and can provoke acute or chronic poisoning. One chronic effect is the so-called black foot disease, occurring mainly in Taiwan, due to relatively high concentrations of arsenic (mainly as arsenite and arsenate) in the drinking water; the symptoms of the disease are degenerations of blood vessels of human extremities, in particular of feet and lower limbs, in

combination with cancerous manifestations of the skin. Sea food, such as crustaceans (e.g., lobster) or molluscs, contains also high concentrations of arsenic, but in the form of arsenobetaine, which is practically completely harmless to man and does not induce either chronic or acute poisoning. Japanese, for example, are well known to eat quite enormous amounts of fish and other sea animals; if all the arsenic would be present as arsenite, many of the people would suffer even from acute poisoning. Thus, the basis for all conclusions dealing with toxicity is the knowledge about the chemical form, in which an element is present in the environment.

Tab. I Human toxicities of arsenic compounds

Species	Formula	Toxicity
arsenic oxide	As_2O_3	lethal dose 1 – 2 mg kg^{-1} body weight
sodium arsenate	Na_3AsO_4	As(V) 3 to 5 times less toxic than As(III)
arsenobetaine	$(CH_3)_3As^+CH_2COO^-$	harmless in gram amounts

Biochemical and biogeochemical information can also be derived exclusively by speciation. Many trace elements play an important role in biochemistry ("essential" trace elements); the only way to reveal their mode of action and their integration in biochemical pathways is the identification and monitoring of the different compounds, in which they occur in living organisms. And only on this basis, biogeochemical cycles of the elements can be sketched, and mobilizations, demobilizations, biotransformations (e.g., methylation) and detoxification processes can be estimated.

Therefore, the overall information we can derive from results obtained by the determination of species as compared to the determination of the total concentration of an element is so much higher and essential for many fields of chemistry and related sciences that in many cases it is an inevitable challenge for analytical chemistry to develop new and sophisticated methods to provide the necessary basic data.

Element Specific Detection in HPLC

What is element specific detection?

Particularly in environmental chemistry, speciation has attracted the main focus of many scientists working in this field, due to the reasons displayed above. Speciation as an analytical concept differs significantly from the routine methods used for determining total concentrations only. In the latter case, when analyzing biological

material (one of the main subjects of environmental analytical chemistry), the primary step is a usually oxidative destruction of the sample, which is achieved either by direct oxidation or by oxidizing agents such as acids either in open systems or in closed vessels (high pressure ashing HPA, microwave digestion, etc.). The reasons are not only to destroy all the organic material, but also to release the element, which is often immobilized in this matrix, and sometimes to convert it into a uniform species which can be easily determined afterwards. Thus, separating procedures are only of minor importance and are usually applied only to get rid of interferences. The ensuing analytical method employs usually some stand-alone device which is operated in batch mode.

The concepts of speciation are diverse and depend on the problem. The simplest design of isolation of one specific species and is ensuing determination turns out practically in many cases to be the most complicated, because of the fact that there are often a variety of chemically similar substances which prevent deliberate isolation of a definite species only. To overcome this problem, the overall procedure is split up into the subtasks of isolation, separation, and determination of the species. Isolation must always be applied when the substances to be determined are not in analyzable form, i.e., usually in most samples except water or other liquid samples. This step is usually one of the most crucial and critical (and, by the way, often neglected by some scientists), since in ideal situation isolation should be quantitative and should not change the chemical composition of the species. The prevailing method is liquid extraction, since other procedures (e.g., volatilization of a volatile, thermally stable species) are only applicable to a very small number of compounds. The primary, extractive isolation process can be combined with some separations and can also separate interferences (e.g., gel permeation chromatography to separate big and small molecules). The analytical procedure consists in the actual separation of the isolated species (gas chromatography, high performance liquid chromatography) with suitable detection, which allows quantitative evaluations. For most problems, high performance liquid chromatography is used because it is easily and widely applicable to separate all different types of substances.

Detectors for HPLC may be arbitrarily classified as non-specific, analyte specific (often called molecule specific) and element specific devices. *Non-specific* detection is usually based on monitoring differential changes of a physical parameter of the mobile phase itself, such as the refractive index, whereas *analyte specific* exploits measurable parameters of the analyte itself. The latter category can range from only semi-specific (e.g., absorption of light in some UV-region) to highly specific detection (e.g., monitoring of the mass of a molecule). In contrast to this, an *element specific* detector responds to all compounds containing a certain element, regardless of the chemical nature of the compound itself, and does not respond to any other substances. This is possible only by exploiting physicochemical properties of the atoms of the element or products derived from them (excited atoms, ions),

which are accessible for convenient monitoring only after complete destruction (atomization) of the compound. Therefore, commonly applied element specific detectors are devices working on the principle of atomization of the compound, i.e., atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES) as well as some types of mass spectrometry (MS).

Why using element specific detection?

The are a few reasons for using element specific detection, which in many cases simplify the analytical procedure considerably or even make determinations of trace element compounds possible. They can be briefly summarized as:

- no interference from co-eluted substances;
- simpler pretreatment of the sample;
- less or no pre-separations required;
- simpler chromatograms;
- simpler chromatography.

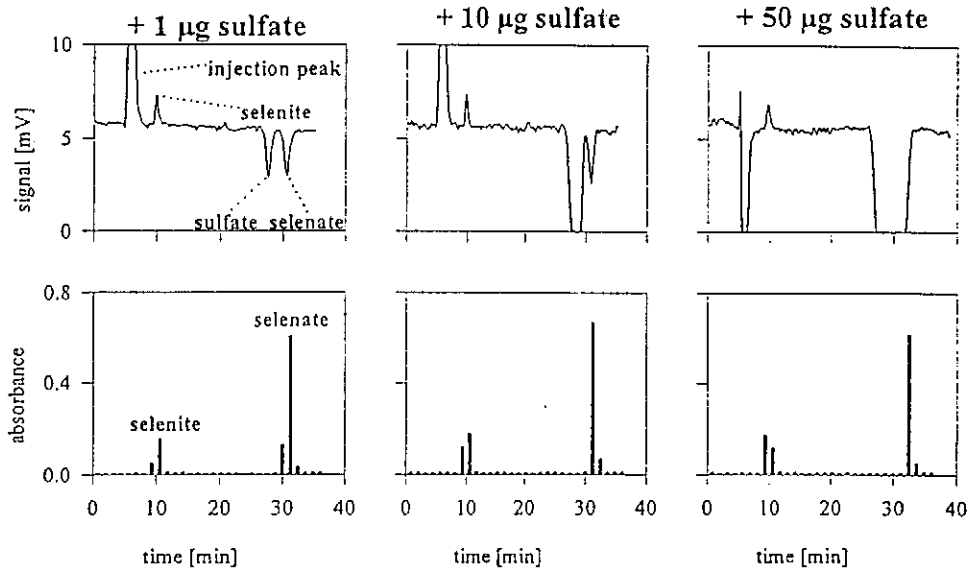

Fig. 1 Comparison of element specific with non-specific detectors. AIEC of selenite and selenate in the presence of increasing amounts of sulphate: 1 µg Se as selenite and selenate, injection volume 100 µl; autosampler sampling volume 20 µl; column: ESA Anion III; mobile phase: 3 mM potassium hydrogen phthalate, pH 7; flow rate: 0.4 ml min^{-1}

As the element specific detector responds only to compounds containing the element of interest, no signal is observed from substances not containing this element. Therefore, all these substances do not interfere with the chromatographic separation (as long as they do not overload the column or interfere chemically). As a result, simple or even no pretreatment of the sample extract is required. Chromatograms can be finally much easier interpreted and evaluated. An example is given in Fig. 1. The chromatograms show the separation of selenite and selenate in the presence of increasing amounts of sulphate. When using conductivity detection, the response of selenate soon becomes completely overlapped by sulphate, whereas with element specific detection it remains unaffected.

But not only evaluation and interpretation of chromatograms becomes simpler, also the chromatography itself. The separation procedures have to be optimized only with respect to the trace element compounds, since other substances do not appear in the chromatograms. Gradient elutions can easily be applied since the baseline is not dependent on the composition of the mobile phase, a problem often encountered with non-specific detectors.

Practical Setup

Instruments which can be used for elements specific detection in HPLC are usually stand-alone devices which are designed for conventional analysis and operated in batch mode (discontinuous, sequential treatment of samples). In order to act as on-line chromatographic detectors, they must be interfaced to the separation system and they must yield data to an output device (recorder, personal computer) more or less continuously, a sometimes challenging task for the analyst. Due to the enormously increasing need of such devices, it may be expected that instruments specifically designed for this purpose will soon be commercially available.

Of course, it is also possible to use element specific detectors off-line after collection of definite volumes of the effluent of the chromatographic column. Restrictions are sometimes imposed, particularly if the required sample volumes must be relatively large for the batch determination. Additionally, intensive labour is required for this type of operation; therefore, in most cases on-line determination is preferable.

The choice of the detector is (apart from the availability of instruments) primarily governed by the problem itself, viz. mainly by the concentration levels at which the trace element compounds occur.

In the following chapters, a few of the most commonly applied element specific detection systems are critically presented and concepts for an effective use are sketched.

Flame Atomic Absorption Spectroscopy (FAAS)

Principle

Atomic spectroscopy in general is a quantitative determination of atoms in gaseous phase by interaction with electromagnetic radiation (light). Compounds are broken down into atoms by thermal energy. Gaseous atoms produced in this way can be determined quantitatively either by absorption of light of characteristic energy, being promoted from the ground energy ground state to an excited one (AAS), or by emission of electromagnetic radiation after excitation by light (atomic fluorescence spectroscopy AFS) or thermal excitation (AES).

In FAAS, a flame produced by the oxidation of a fuel gas (acetylene, hydrogen) with an oxidant (air, oxygen, dinitrogen monoxide N_2O) serves as the atomization source. The sample (applied as a solution) is sucked via a capillary into a nebulizer, and the resulting aerosol is introduced into the flame. There occur different processes there (evaporation of the solvent, evaporation of the remaining salt particles, atomization of molecules) yielding, under optimized conditions, atoms prevailingly in the ground state. Characteristic light from a hollow cathode lamp or an electrodeless discharge lamp containing the element is sent through the bulk of gaseous atoms and the diminuation of its intensity is measured.

Interfacing and Data Handling

Interfacing of an HPLC system to a flames atomic absorption spectrometer is simple and consists of a connection between the effluent capillary of the chromatographic column with the sample capillary of the nebulizer, which continuously uptakes the sample solution. The optimum uptake rate ranges between 2 and 4 $ml\ min^{-1}$, therefore, the flow rate of the chromatographic pump should be adjusted to these values; but even slower flow rates are acceptable.

A compact commercial routine device may impose some problems concerning data handling, because they do not provide continuous accessibility of data. Therefore, attention should be paid that either necessary software is available (time resolved software), or data are reasonably quickly accessible in digital (communication port) or analogous form. In the latter case, the analog signal can be directly taken as input for a recorder, an integrator or for standard chromatographic software. Analog signals can be easily digitized (and thereupon handled by computers) with the aid of AD (analog-to-digital), ADDA (analog-to-digital digital-to-analog) converters or so-called mutlifunctional boards. They facilitate simple registration of data and handling by self-designed and specifically tailored computer programs. AD-converters should have at least 12 bits resolution (meaning that the total output signal range is recorded in 2^{12} units. Stand-alone converters are

preferable to boards directly inserted into personal computers, since they are less susceptible to electric noise.

Applicability, Advantages and Disadvantages

All various types of chromatography (normal phase, reversed phase, ion pairing, ion chromatography) with various mobile phases (from purely aqueous to highly organic) can be combined with FAAS for element specific detection (Table II). Compounds of all elements which can be determined by conventional FAAS can also be determined by the hyphenated technique. The main disadvantage is that the detection limit for most elements is rather high (ppm range), which limits practical applicability significantly. But it is an excellent method to establish and optimize chromatographic methods, before they are applied with more sophisticated detectors. The basic investment costs are low, and so are the costs of operation.

Tab. II Advantages and disadvantages of some element specific detectors

Method	Detection Limits	Advantages	Disadvantages
FAAS	ppm	cheap easy coupling to HPLC no chromatographic limitations	high detection limit
GFAAS	medium ppb	good detection limits no chromatographic limitations	discontinuous operation difficult coupling and control long analysis time problems with evaluation
ICP-MS	sub-ppb	excellent detection limits easy coupling to HPLC multielement monitoring applicable to many samples	expensive chromatographic limitations residual ionic effects

Examples

Figure 2 shows the separation and determination of selenium compounds by cation- (CIEC), anion exchange (AIEC) and reversed phase ion pair partitioning chromatography (RPIPPC); Fig. 3 shows the separation of chromium species when monitoring the reaction of chromate with oxalate by RPIPPC. Immediately after adding chromate to oxalate, three different complexes of chromium(III) with oxalate (mono-, di- and trioxalato-complex) and Cr(VI) can be detected; after some time (60 min) Cr(VI) disappears and the trioxalate complex is the prevailing species.

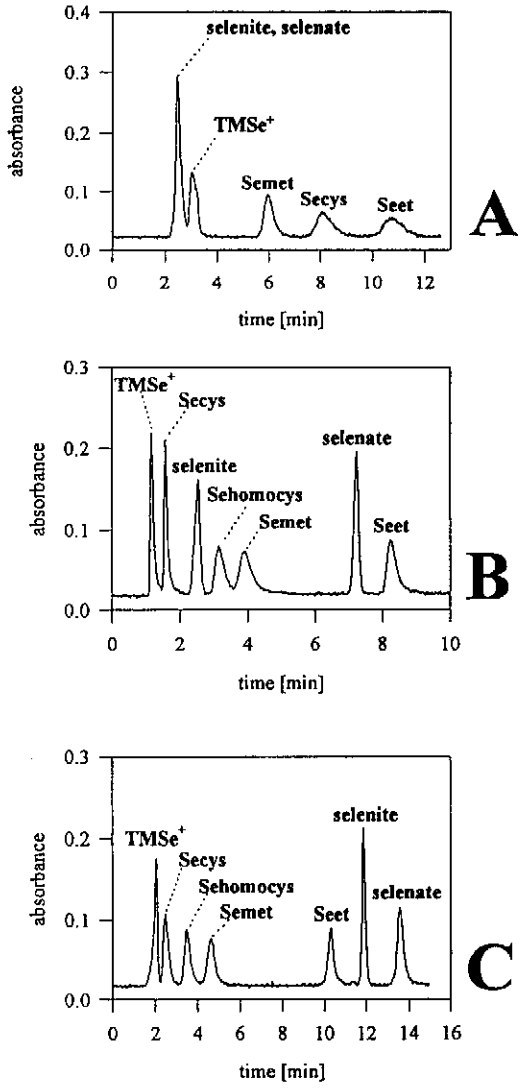

Fig. 2 Separation of Se-compounds using various types of chromatography and FAAS detection ($TMSe^+$ – trimethylselenonium iodide, Secys – selenocystine, Sehomocys – selenohomocystine, Semet – selenomethionine, Seet – selenoethionine): 10 μg Se for each compound, injection volume 100 μl. A – cation exchange chromatography; column: Hamilton PRP-X200, mobile phase 100 mM nitric acid in 9:91 (v/v) isopropanol/water; flow rate: 1 ml min^{-1}; B – anion exchange chromatography; column: ESA Anion III; mobile phase: 2 mM potassium hydrogen phthalate (KHP), pH 9.0, after 2.5 min 12 mM KHP, pH 9.0 in 20 : 80 (v/v) methanol/water; flow rate 2 ml min^{-1}; C – reversed phase ion pair partitioning chromatography; column: Hamilton PRP-1; mobile phase: 2 mM tetrabutyl ammonium dihydrogen phosphate (TBAP), after 2 min 2 mM TBAP in 50 : 50 (v/v) methanol/water; flow rate: 1 ml min^{-1}

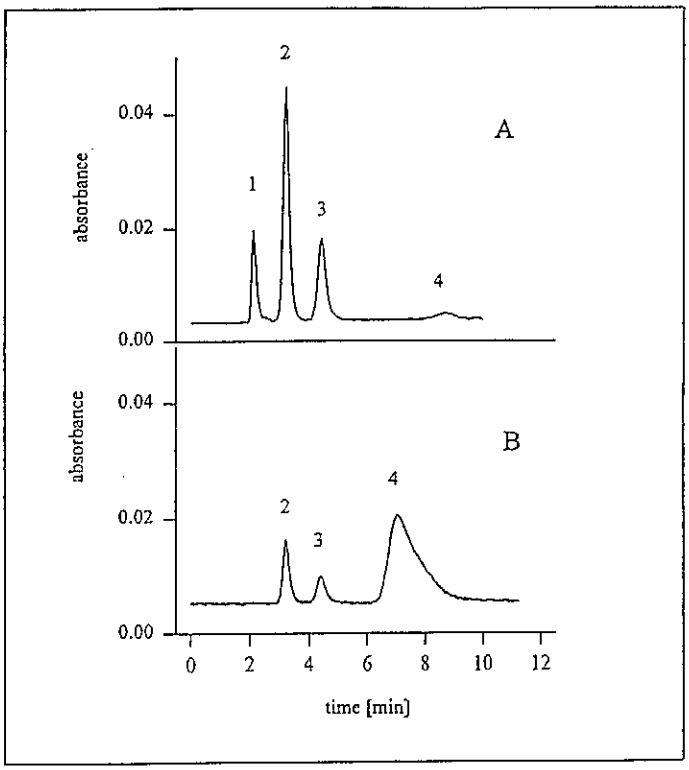

Fig. 3 Separation of chromium species occurring during reaction of chromate with oxalate (ox): A – solution immediately after mixing; B – after one hour; 1 – $[Cr(ox)(H_2O)_4]^+$, 2 – $[Cr(ox)_2(H_2O)]^-$, 3 – CrO_4^{2-}, 4 – $[Cr(ox)_3]^-$; injection volume 100 µl; column: Hamilton PRP-1; mobile phase: 1 mM TBAP in 1 : 1 (v/v) methanol/water, pH 6.0; flow rate 1 ml min^{-1}

Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)

Principle

This method, also known as electrothermal AAS (ETAAS), is similar to FAAS, only the atomization method is different. In batch operation, a sample (10–100 µ l) is introduced into a small graphite cuvette, which is ensuingly heated up (up to 3000 °C) by electric current, so that all those processes occur in the cuvette which also occur in the flame. By deliberate heating it is possible to split up the processes, which are occurring more or less simultaneously in the flame, into sequential events. Thus, heating is not performed immediately, but in usual three time domains—drying, ashing, and atomization. During drying (for usually 10 to 20 s), the solvent

is evaporated leaving a dry residue of the sample. During a subsequent elevation of the temperature (ashing, similar period of time as drying), this residue is converted into a homogenous compound and organic substances are volatilized. Finally, the current is increased and atomization occurs at high temperatures (3 s). After cooling down, a new sample can be injected into the cuvette. Injection can be done manually, but is usually performed by an autosampler, which guarantees better mechanical reproducibility and, therefore, better results. Thus, one complete analysis requires about 1.5 to 2 minutes, until all actions of the instrument (autosampler injection, drying, ashing, atomization, cooling) are executed.

Interfacing, Control and Data Handling

The main problem, when interfacing GFAAS with HPLC, is the discontinuous mode of operation of the analytical device and the relative long period of time required for obtaining one data point.

Interfacing is done by means of a flow through cell of a volume of about 50 µl, where excess of effluent from the chromatograph is removed by a water aspirator pump [2–4]. The flow through cell is positioned under the nozzle of the autosampler, which collects repetitively volumes of 10 or 20 ml and injects them into the graphite furnace.

A few attempts were made [2–4] to reduce the unacceptably long acquisition time for one data point on the chromatogram (1.5 – 2 min). The best way seems to use a modular instrument and to control its actions by a specifically designed software.

Compact routine instruments have preset cooling periods for the cuvette and autosampler action, which take place in a sequential order and, since not being externally controllable, are rather unsuitable for element specific detection. Improvement can be achieved by using devices which consist of modules (autosampler module, power module for heating, optical module for measurement) which interact by handshaking with TTL (transistor-to-transistor logic) signals, i.e., signals are used to activate a module and to indicate its state of activity (idle or active). Practice has shown that the periods usually preset for the autosampler action and for cooling are too long and can be shortened significantly; additionally, the ashing period can be omitted during the atomization procedure, thus obtaining data acquisition intervals of about 30 – 40 s (Fig. 4). Control of the modules is achieved by interrupting the handshake connections between the autosampler, power and control module, and by activating the units and monitoring their state of activity by the digital ports of a multifunctional board and a personal computer [4]. Thus, the autosampler can be started even during the atomization of the previous sample, and the heating of the new sample can be started before the full cooling period has expired. At the same time, the analog signal can be registered by the analog port of

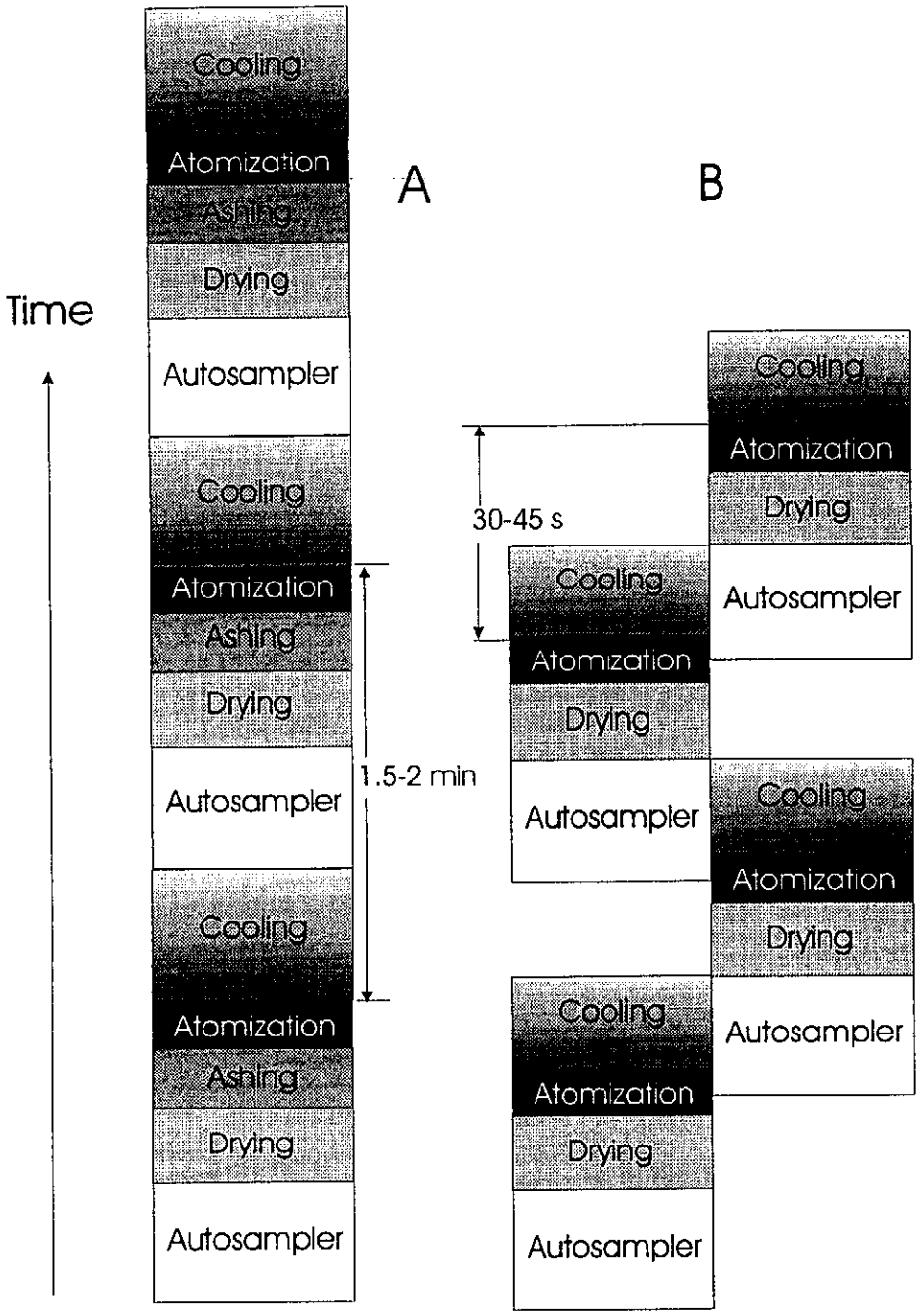

Fig. 4 Schematic representation of instrument action in batch analysis (A) and for element specific detection (B)

the multifunctional board. The schematic flow chart of a software designed for this purpose is sketched in Fig. 5.

The evaluation of the resulting chromatographic peaks can be done in different ways. The worst method is the peak height approximation, since only by chance tops of the peaks are encountered. A better approach is to sum the signals within peaks, the best results being obtained by synthesizing Gaussian curves over the peaks and integrating the area under the fitted graph.

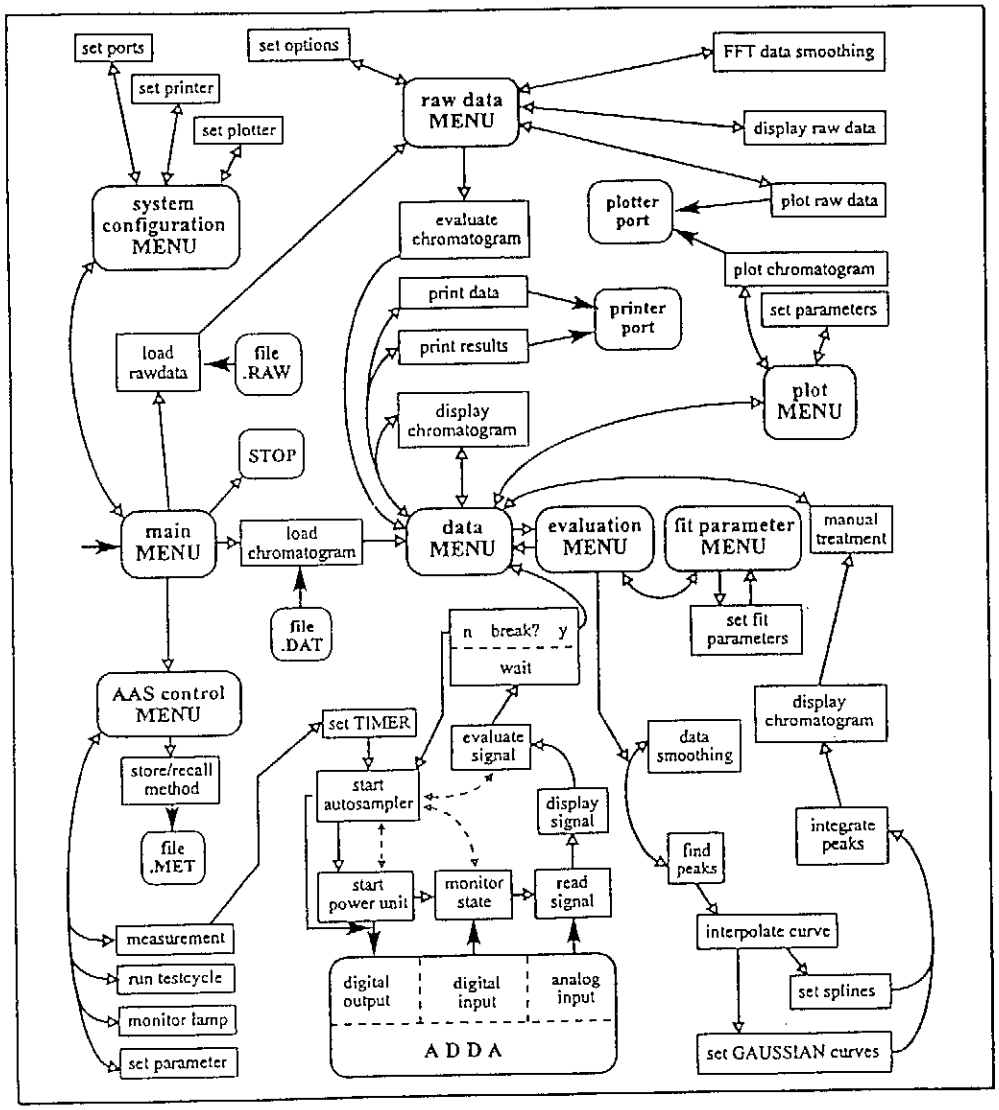

Fig. 5 Schematic flow chart of a control program for a GFAA spectrometer

For most elements, GFAAS has usually a significantly lower detection limit (medium to low ppb range) than FAAS and can be employed for the analysis of many biological samples. In principle, all the various types of chromatography applicable with FAAS can also be used with GFAAS. But on-line detection presents many problems and disadvantages, which makes the application unfavorable and unattractive.

The main disadvantage is that even when applying sophisticated control programs the acquisition time for one signal is unfavorably high. Usual chromatographic methods of achieving narrow peaks in a short time are not applicable. Broad peaks are better reproduced in the chromatogram than sharp ones, which inferes the latent danger of them not being monitored at all. As a consequence, one chromatographic run requires long times (40 min or even considerably more) at low flow rates (e.g., 0.2 ml min^{-1}). Quantitative evaluation of peaks is problematic, since they are represented by a few data points only. Besides that, interfacing is not so simple, and much effort must be made to control the device efficiently. A reasonable alternative is off-line batch detection after collecting fractions of the chromatographic effluent.

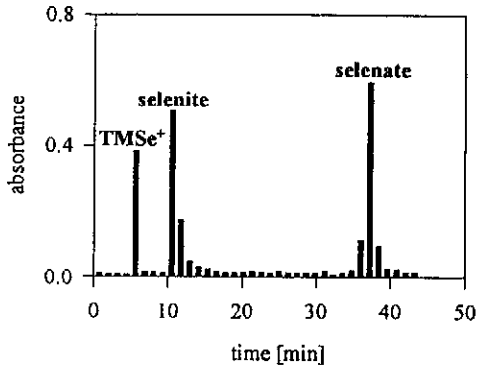

Fig. 6 Anion exchange chromatographic separation of trimethylselenonium iodide, selenite, and selenate using GFAAS detection: 100 ng Se for each compound; injection volume 100 µl; autosampler sampling volume 20 µl; column: ESA Anion III; mobile phase: 3 mM KHP, pH 7; flow rate: 0.4 ml min^{-1}

Examples

Examples for separating three selenium compounds by AIEC and detecting them with GFAAS are given in Figs 1 and 6. The latter example indicates a possibility

how to increase the sensitivity of GFAAS. Matrix modification for the graphite furnace technique is a common procedure to lower the detection limit of some elements significantly. It means that some additives to the sample can increase the signal of the analyte. In most cases matrix modifiers were found empirically, whereas their way of action is still unclear. Selenium gives significantly better absorption signals in the presence of nickel salts, when the latter is added as a matrix modifier. Such effects can even be exploited when using GFAAS for element specific detection: the modifier is a constituent of the mobile phase and thus produces significantly better signals. But, of course, the common problems for on-line detection still remain.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Principle

Plasmas are a good atomization source with temperatures of up to 10000 K, which are significantly higher than in flames (about 6000 K). Plasmas represent a highly energetically excited state of a gas with mainly ions prevailing. Whereas the energy in flames is obtained by an oxidation process, formation of plasmas is based on a different principle. Commonly, the carrier is a noble gas (usually argon), which is in different ways excited by electric current. When substances are brought into the plasma they are not only atomized but also thermically excited and ionized, due to the high temperature. Therefore, plasmas are an excellent atomization source for atomic emission spectroscopy, but they can also act as ion sources for mass spectrometry.

One of the most common types of plasmas is the inductively coupled plasma (ICP; Fig. 7). The torch consists mainly of a quartz tube through which argon is passed. Ignition is performed by an electric discharge, which produces primary ions and electrons. An induction coil (therefore the term "inductively coupled") consisting of three to four windings of a thick copper wire around the end of the tube is connected with a powerful radio frequency generator and effects vigorous movement of the charged particles caused by the fast change of polarity of the electric field. The agitated ions collide with neutral argon atoms and ionize them in turn during collision. When leaving the tube the excited atoms and ions emit light and recombine with electrons yielding a "plume" which is similar to a combustion flame in its appearance. A concentric capillary in the middle of the quartz tube allows introduction of nebulized samples (analogously to FAAS) into the plasma. As with FAAS, the sample undergoes a similar fate as in a flame with FAAS with the significant difference that excitation and ionization of the atoms after the breakdown of the molecules dominate. The uptake of sample by the nebulizer is quite similar to that of FAAS, the typical uptake rate being somewhat lower (about

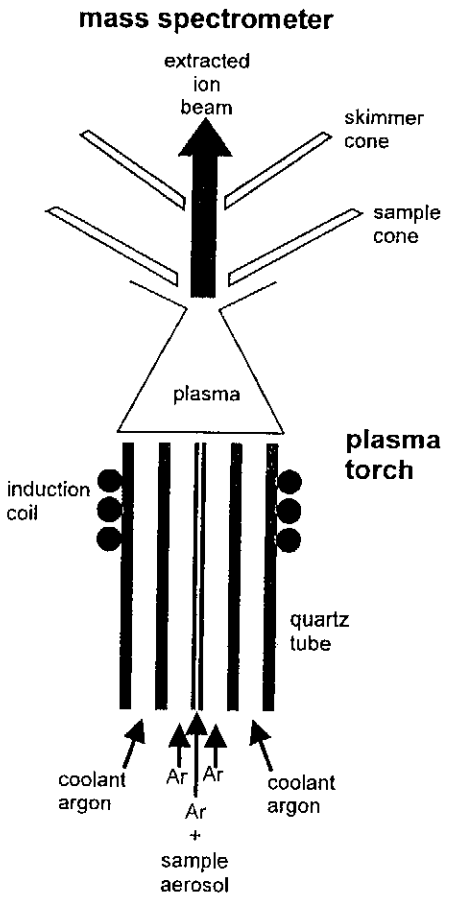

Fig. 7 Sketch of ICP-MS instrumentation

1 ml min^{-1}).

A plasma generated by inductive coupling can serve as an ion source for mass spectrometry. In contrast to conventional MS, only ions of atoms can be monitored (not molecules or fragments) since the high temperature provokes complete disintegration of the molecules. In conventional ICP-MS a quadrupole mass spectrometer is attached to the plasma. Since MS requires pressures of around 10^{-6} mbar, whereas the plasma is operated at ambient pressure, a stepwise reduction of pressure with simultaneous extraction of ions is effected via two cones with a concentric drill holes (orifices) in combination with effective vacuum pumps.

A quadrupole MS applies an electrostatic quadrupole electric field on four symmetrically arranged electrically conductive rods where oposite rods have the same polarity. An alternating field is superimposed, causing osscillation of entering ions. Resonance conditions of oscillations (resulting in detection) are dependent on

one hand on the mass-to-charge ratio and on the other on the voltage ratio of direct and alternating field and on the frequency. Nowadays sophisticated instruments are available using a high resolution mass spectrometer instead of a quadrupole MS with resolutions down to 0.001 atomic mass unit.

ICP can also be used with AES (inductively coupled plasma optical emission spectroscopy, ICP-OES) as an element specific detector, but its detection limit is then rather high (ppm to medium ppb level).

Interfacing, Data Handling

Interfacing of ICP-MS to HPLC is as simple as with FAAS and is done by connecting the exit capillary of the chromatographic column with the uptake capillary of the nebulizer. The flow rate of the chromatographic system should be set around 1 to 1.5 ml min^{-1}, which corresponds to optimal uptake by the nebulizer. Registration of the chromatogram is done by monitoring a mass of an isotope of an element. Simultaneous detection of more than one element is possible by monitoring more channels of different masses. Control of the instrument and registration of data is performed by personal computers and rather complex software. For monitoring chromatograms time-resolved software is necessary which is commercially available for most instruments.

Applicability, Advantages and Disadvantages

ICP-MS as an element specific detector is applicable for many analytical tasks in environmental chemistry and yields excellent results. Its main advantage is a very low detection limit for many elements (usually in the sub-ppb range) and the possibility of simultaneous multielement monitoring.

There are limitations with respect to the composition of the mobile phase, in particular should the salinity not exceed ca 20 mM (otherwise the orifice of the sample cone will soon be obstructed). Additionally should the concentration of organic solvents not be higher than 20 %, otherwise explosion-like occurrences in the plasma could damage the instrument, or the plasma could be extinguished. Other limitations are encountered when using conventional mass spectrometers. Due to residual ionic effects, a few masses cannot, or only with difficulty can, be monitored, primarily masses 40 ($^{40}Ar^+$) and 80 ($^{40}Ar_2^+$), which is a disadvantage when isotopes of such masses are the most abundant of an element (e.g., ^{80}Se). But also due to formation of charged aggregates between atoms produced from components of the chromatographic effluent and/or argon atoms, interferences can occur and deterior determinations, e.g., $^{40}Ar^{35}Cl^+$ (interferes with ^{75}As), $^{35}Cl^{16}O^+$ (interferes with ^{51}Cr), $^{37}Cl^{16}O^+$ (interferes with ^{53}Cr), $^{40}Ar^{12}C^+$ (interferes with ^{52}Cr) etc.

Hopefully, further developments have already, or will in the near future, eliminate a few of these disadvantages; use of high resolution mass spectrometers will overcome restrictions due to residual ionic effects, and specially designed nebulizers can efficiently remove most of the solvent from the aerosols before their entrance into the plasma.

Examples

Figure 8 displays an AIEC separation of selenium species using ICP-MS for detection. In Fig. 9 chromatograms can be seen obtained with human urine of a test person who was administered selenium (as seleno-aminoacids). In the urine trimethylselenonium was found, which demonstrates biotransformations taking place in the human body [5–9].

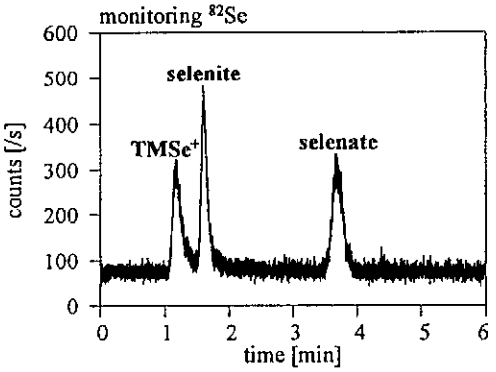

Fig. 8 AIEC separation of selenium compounds using ICP-MS detection: 10 ng Se for each compound; column; ESA Anion III; injection volume 100 µl; mobile phase 5 mM KHP, pH 7; flow rate: 1 ml min^{-1}

Figure10 shows two examples for the determination of chromium in tap water and in coal fly ash [10].

Prospects

Element specific detection in combination with chromatographic separations is already an indispensable tool in inorganic and bio-inorganic analysis nowadays, particularly in environmental chemistry for the determination of trace element compounds. As is reflected by the enormously growing number of publications per

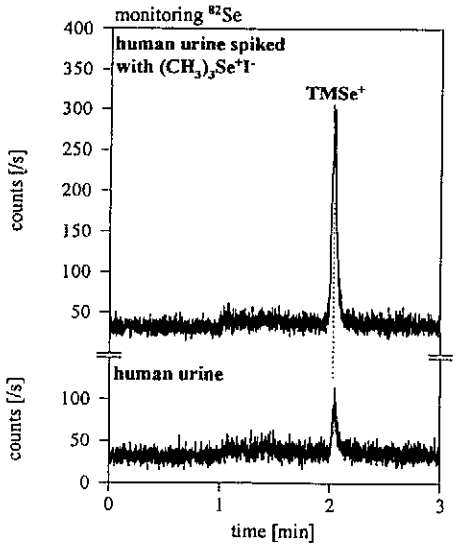

Fig. 9 Identification of trimethylselenonium ions in human urine using CIEC with ICP-MS detection: mass monitored 82; injection volume 100 μ l; spike: 50 μg l^{-1} Se as $TMSe^+$; column: Hamilton PRP-X200; mobile phase: 20 mM nitric acid in 2 : 98 (v/v) methanol water; flow rate: 1.5 ml min^{-1}

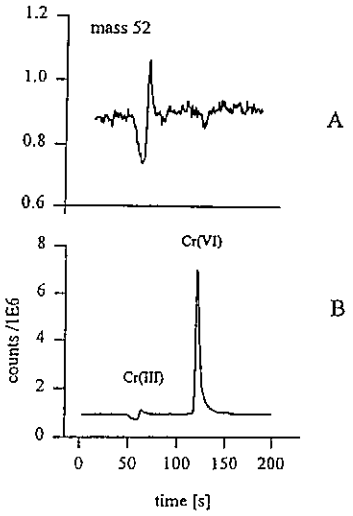

Fig. 10 Speciation of chromium by RPIPPC with ICP-MS detection in tap water (A) containing 0.6 ppb Cr(III) and in an aqueous extract of coal fly ash (NIST SRM 1632a; B): mass monitored 52; injection volume 100 μl; column: Hamilton PRP-1; mobile phase: 2 mM tetraethylammonium phosphate, pH 3.5; flow rate 1.5 ml min^{-1}

year, the need for it will increase drastically in the coming years.

Nowadays, many standardized procedures for the determination of total concentrations of elements are established. In the future, one of the main tasks of analytical chemistry will be to develop also standard procedures for the determination of trace element compounds along with providing standard reference materials, which are certified not only for concentrations of total elements, but also for various species. These facts, together with commercial availability of routine instruments, will facilitate "speciation" as routine work in analytical laboratories. Thus, analytical chemistry will further contribute to a better understanding of the role of elements in the environment, its interactions, all to the benefit of man.

References

1. Irgolic K.J.: Chem. Speciation Bioavail. **1**, 127 (1989).
2. Brinckman F.E., Blair W.R, Jewett K.L, Iverson W.P: J. Chromatogr. Sci. **15**, 493 (1977).
3. Stockton R.A, Irgolic K.J.: Int. J. Envir. Anal. Chem. **6**, 313 (1979).
4. Kölbl G., Kalcher K., Irgolic K.J.: J. Autom. Chem. **15**, 37 (1993).
5. Kölbl G., Kalcher K., Irgolic K.J.: Appl. Organomet. Chem. **7**, 443 (1993).
6. Kölbl G., Kalcher K., Irgolic K.J.: Anal. Chim. Acta **284**, 301 (1993).
7. Kölbl G., Krachler M., Kalcher K.: *Proceedings of the 5th International Symposium on Uses of Selenium Tellurium* (Brussels, May 8 – 11, 1994), p. 291.
8. Kölbl G., Lintschinger J., Kalcher K., Irgolic K.J.: Mikrochim. Acta **119**, 113 (1995).
9. Kölbl G., Kalcher K., Irgolic K.J. in: *Main Group Elements and Their Compounds. Perspectives in Materials Science, Chemistry & Biology* (V.G. Kumar Das, ed.), pp. 161–172, Narosa Publishing House, 1996.
10. Lintschinger J.L., Kalcher K., Gössler W., Kölbl G., Novic M.: Fresenius J. Anal. Chem. **351**, 604 (1995).