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Atomic absorption spectrometry in pharmaceutical analysis*

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Abstract: A short review of the principles of atomic absorption spectrometry (AAS) — sources, atomizers, background correction, instrumentation, sample preparation, sensitivity, limit of detection, etc. — is given. The technique can be applied for impurity tests for trace metals as well as for assay of various commonly occurring elements in pharmaceuticals. The application of AAS in pharmaceutical analysis is illustrated with 17 examples.

Keywords: *Atomic absorption spectrometry; application in pharmaceutical analysis.*

Introduction

It was in October 1954 that Alan Walsh of the Commonwealth Scientific and Industrial Research Organization, Australia submitted his paper [1] in which he discussed the factors governing the relationship between atomic absorption and atomic concentration, and the experimental problems involved in making atomic absorption measurements. At about the same time Alkemade and Milatz had arrived [2] independently at the atomic absorption method. Walsh, giving his personal reminiscences on the discovery of this technique, said "early in 1952 I began to wonder why, as in my experience, molecular spectra were usually obtained in absorption and atomic spectra in emission. The result of this musing was quite astonishing: there appeared to be no good reason for neglecting atomic absorption spectra; on the contrary, they appeared to offer many vital advantages over atomic emission spectra as far as spectrochemical analysis was concerned. There was the attraction that absorption is, at least for atomic vapours produced thermally, virtually independent of the temperature of the atomic vapour and of excitation potential. In addition, atomic absorption methods offered the possibility of avoiding excitation interference, which at that time was thought by many to be responsible for some of the interelement interference experienced in emission spectroscopy when using an electrical discharge as light source". Walsh described his first experiment as follows: "The sodium lamp was operated from 50 cycles/sec. and thus had an alternating output so that it was not necessary to use a chopper. The D lines from this lamp were isolated — but not resolved from each other — by means of a direct vision spectroscope and their

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intensities were measured by means of a photomultiplier tube, the output from which was recorded on a cathode ray oscillograph. With the slit-width used the signal gave full-scale deflection on the oscillograph screen. A Meker flame was interposed between the sodium lamp and the entrance slit of the spectroscope. When a solution of sodium chloride was atomised into the air supply of the flame the signal at the oscillograph was reduced to zero. The principle of atomic absorption was therefore established" [3]. This is how atomic absorption spectrometry, AAS, started 30 years ago. It has since become one of the most widely used quantitative analytical methods with high sensitivity, selectivity and precision for the analysis of almost 70 elements in various fields including pharmacy. A short account of the various aspects of AAS is given here [4–7].

Basic Principles

If an atomic vapour containing free atoms of an element in the ground state is illuminated by a light source that radiates light of a frequency characteristic of the element present in the vapour, the neutral free atoms absorb the resonant frequency. The beam of light is attenuated due to resonance absorption. The energy absorption is virtually a direct function of the number of atoms in the absorbing path and approximately proportional to the total number of atoms in ground state. According to the Boltzmann relationship there is a very high proportion of ground state to excited state atoms at temperatures of about 3000 K, providing two particular advantages of atomic absorption. It gives a far better sensitivity than emission, and absorption measurements are less dependent upon short-term flame temperature variations than emission.

A factor which also has practical implications is the wavelength of a resonance line which is inversely proportional to its energy. The ratio of the number of atoms in the excited state to the number in the ground state decreases exponentially with increasing resonance line frequency. Thus it is expected and found that elements whose resonance lines occur at higher wavelengths are more sensitive in emission than those whose resonance lines are at low wavelengths. Lithium (670.7 nm) and sodium (589.0 nm), for example, are more sensitive in emission than copper (324.8 nm) or magnesium (285.2 nm) while zinc (213.9 nm) is very insensitive indeed in emission. The natural width of an atomic spectral line is of the order of 10^{-4} Å, but this is broadened by Doppler, electric field and pressure effects. The half-width of the resonance lines lie in the range 0.005–0.05Å. The resolution of the usual instruments is not sufficient to measure the profile of such a line with accuracy. These difficulties are normally avoided by using sharp line sources which produce very narrow lines.

Light Sources

The primary light sources in AAS may be classified as continuous emitters and discrete emitters. The first type produces a continuous emission over a considerable range of the spectrum; the second is characterized by a discrete spectrum consisting of narrow, separated lines usually characteristic of only one element. Continuous light emitters such as hydrogen, tungsten and high-pressure xenon-arc lamps are hardly used as light sources in analytical AAS, because the absorption lines are very narrow and the brightness of the continuous sources is too low for satisfactory measurement of the absorption. Besides, spectral interferences are large as the source is not element-specific. Microwave-excited electrodeless discharge tubes, hollow-cathode lamps, high intensity lamps and flames are

the principal discrete emitters. Narrow emission lines are obtained by the use of hollow-cathode lamps. Under optimum conditions it is not necessary to use slit-widths as narrow as the half-width of the absorption line: it is sufficient if the spectrometer isolates the required emission line from other lines emitted by the same source. The emission of hollow-cathode lamps consists of a very steady, brilliant and clean spectrum showing very narrow lines of the element forming the cathode.

Types of Atomizers, Flame and Flameless Techniques

In order to make measurements in atomic absorption it is necessary to devise an experimental assembly which will convert the material under examination as efficiently as possible to a population of ground state atoms. The most convenient, stable and economic source of the production of atomic vapour remains the combustion flame. Fuel-oxidant mixtures are commonly used to produce a range of temperatures from *ca.* 2000 to 3000 K. Fuel gases include propane, hydrogen and acetylene; oxidants include air, nitrous oxide and sometimes oxygen mixed with an inert gas such as nitrogen or argon.

Non-flame atomization techniques include furnaces and electrically induced plasmas. The L'vov furnace [8], the resistance-heated graphite-tube furnace devised by Massmann [9], the heated rod carbon filament, cathodic sputtering and the plasma torch are some of the flameless devices used for atomization, among which the resistance-heated graphite-tube furnace is widely used. Non-flame atomization techniques are characterized by their good detection limits of elements in the range of 10^{-9}–10^{-12}g, generally not reached with flames.

Background Correction

Scatter and background absorption enhances the measured absorbance values. Scatter is analogous to turbidity in molecular spectrometry and is the result of the presence of small solid particles in the path of the resonance beam. Such solid particles may be caused by the flame's inability to vaporize a high dissolved solids content of the sample solution or may be due to the formation of carbon particles in the flame itself. This scattering effect is dependent on the wavelength and according to Rayleigh theory a sharp increase of the background absorption due to light scattering is observed towards lower wavelengths. Background absorption covers a large range of wavelengths, whereas atomic absorption takes place in a very narrow wavelength range, *ca.* 0.02 Å. Background absorption can in principle be corrected by using a blank solution of exactly the same composition as the sample but without the specific element to be determined. This is frequently impracticable. Another method of background correction is the use of two different light sources, the hollow-cathode lamp that measures the total absorption (i.e. elemental + background) and a deuterium lamp with a continuum emission that measures the background absorption [10]. Background absorption is corrected by subtracting the deuterium lamp signal from the hollow cathode lamp signal. Still another method for the correction of background absorption is the Zeeman effect — atomic absorption caused by the Zeeman splitting of the absorption line in a magnetic field [11]. This is particularly useful when the background absorption shows fine structure, for example in the electronic excitation spectra of molecules and radicals. Apart from background absorption correction there are chemical, matrix, ionization and spectral interferences in the atomic absorption measurements. These are compensated by (for

example) the addition of suitable electrolytes, using the blank solution of same physical properties as the sample matrix, and applying single element instead of multielement hollow cathode lamps.

Instrumentation

The basic atomic absorption spectrometer uses light from the source, generally a hollow-cathode lamp, which passes through the flame, (air–acetylene or N_2O–acetylene) into which the sample solution is sprayed as a fine aerosol. The region of the spectrum in the immediate neighbourhood of the resonance line to be measured is selected by the monochromator. The isolated resonance line falls on to the detector, a photomultiplier, the output of which is registered. The intensity of the resonance line is measured with and without the sample passing into the flame. The difference between these readings is a measure of the absorption and proportional to the amount of the element being determined. In order to avoid measuring the emission from the excited atoms in the flame at the same wavelength, the source-lamp intensity is modulated mechanically or electrically and the amplifier is tuned to the same frequency. Consequently the continuous component of radiation signal originating from the flame is not measured. Background correction of the absorption is performed with a deuterium lamp where it is required. Figure 1 is a sketch of a simple atomic absorption spectrophotometer.

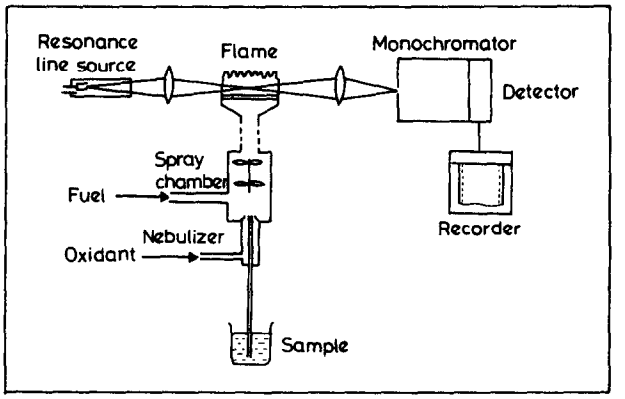

Figure 1
Simple AAS apparatus.

Sample Preparation, Sensitivity, Limit of Detection

Samples in AAS can be prepared in aqueous and non-aqueous solutions. The analytical sensitivity of an element in AAS is defined as the concentration, usually in parts per million, which will absorb 1% of the incident resonance radiation, i.e. which has an absorbance of 0.0044. The detection limit in atomic absorption is the concentration corresponding to twice the standard deviation of a series of not less than ten readings taken close to the blank level. Table 1 gives the detection limits in flame and flameless AAS for a large number of pharmaceutically interesting elements.

Concentrations are determined either using a calibration curve constructed with standards (dissolved possibly in the same blank solution as the sample), or by applying the standard-addition method, in which a known amount of the specific element is added in increasing concentrations to the sample solution.

Table 1
Detection limits with flame (μg/ml) and flameless AAS (absolute in g) respectively

Element	Flame (μg/ml)	Hydride-technique (g)	Graphite-tube-furnace (g)
Mo	(N_2O/C_2H_2) 0.03	—	1×10^{-10}
Na	(air/C_2H_2) 0.002	—	1×10^{-11}
Ni	(air/C_2H_2) 0.02	—	1×10^{-9}
Pb	(air/C_2H_2) 0.02	—	1×10^{-10}
P	(N_2O/C_2H_2) 100	—	—
Se	(Argon/H_2) 0.1	1×10^{-10}	1×10^{-9}
Zn	(air/C_2H_2) 0.002		2×10^{-12}
Au	(air/C_2H_2) 0.02	—	5×11^{-11}
Pd	(air/C_2H_2) 0.02	—	5×10^{-10}
Zr	(N_2O/C_2H_2) 5	—	—
Si	(N_2O/C_2H_2) 0.1	—	—
Al	(N_2O/C_2H_2) 0.05	—	2×10^{-10}
Sn	(air-C_2H_2) 0.2 N_2O/C_2H_2)	5×10^{-10}	—
Tl	(air/C_2H_2) 0.025	—	2×10^{-11}
Ag	(air/C_2H_2) 0.002		2×10^{-11}
As	(Argon/C_2H_2) 0.01	1×10^{-10}	1×10^{-10}
Ba	(N_2O/C_2H_2) 0.02	—	—
Ca	(air/C_2H_2) 0.01	—	1×10^{-10}
Cd	(air/C_2H_2) 0.001		1×10^{-12}
Co	(air/C_2H_2) 0.01	—	1×10^{-10}
Cr	(air/C_2H_2) 0.003	—	1×10^{-10}
Cu	(air/C_2H_2) 0.002	—	1×10^{-11}
Fe	(air/C_2H_2) 0.01	—	2×10^{-11}
Hg	(air/C_2H_2) 0.5	1×10^{-9}	2×10^{-10}
K	(air/C_2H_2) 0.005	—	2×10^{-11}
Li	(air/C_2H_2) 0.001	—	2×10^{-11}
Mg	(air/C_2H_2) 0.0005	—	1×10^{-12}
Mn	(air/C_2H_2) 0.002	—	2×10^{-11}

AAS has been included as an analytical technique in the *European Pharmacopoeia*, volume III, BP 80, NF XV and the *Swiss Pharmacopoeia*. The application of AAS in pharmaceutical analysis can be illustrated with the following examples from our laboratory [12]. The instrument parameters (wavelengths, gas mixtures, slit widths, etc.) for each element are taken from instrument manufacturers' catalogues.

Examples of AAS Applications

1. *Determination of traces of potassium in NaCl and sodium in KCl.* The *European Pharmacopoeia* has limited the content of sodium in KCl and of potassium in NaCl to 0.1%. Innumerable measurements showed that the content of these elements is generally less than 0.01%.

2. *Determination of alkaline and earth-alkaline metals in electrolytes for infusion.* Chemical interferences in calcium determinations are eliminated through the addition of lanthanum nitrate. While using a N_2O–acetylene flame a large slit width should be avoided due to the presence of an intense CN emission signal between 410 and 422 nm. The presence of silicon and aluminium affects magnesium absorption, but these elements are generally not present in electrolytes. Alkali metals may also be determined at insensitive wavelengths, e.g. potassium at 401.7 nm and sodium at 330 nm.

3. *Trace elements in multivitamin formulations.* $CuSO_4$ (0.5 mg), $CoCl_2$ (0.4 mg), ZnO (0.06 mg) and $MnSO_4$ (0.1 mg) are generally present as important trace elements for body metabolism in multivitamins. They can be easily determined either with flame or flameless AAS. Spectral interferences should be taken into account. For example, the cobalt analysis would be affected by nickel while using a dual element Co/Ni hollow cathode lamp.

4. *Aluminium assay in biological samples such as rat-fodder or antacids.* Aluminium can be determined in the range of 10–50 μg/ml in aqueous solutions in a N_2O–acetylene flame after a wet-chemical decomposition or acid extraction of the samples.

5. *Zinc in insulin and cobalt in vitamin B12.* The zinc content of insulins, which should be about 0.3%, can be checked through AAS after acid decomposition of the sample. Vitamin B12 can be assayed indirectly through cobalt (4.34% Co in cyanocobalamin) determination. This analysis is not very specific as vitamin B12 decomposition products also contain cobalt.

6. *Tin assay of an anthelmintic powder.* Worm infestations in animals are treated with an anthelmintic powder which contains organic tin compounds such as dibutyl-*n*-tindilaurate. Sn can be determined in a N_2O–acetylene flame in the range 100–400 μg/ml after chemical decomposition of the sample. An ionization interference is observed in this flame for tin which is eliminated through the addition of alkali metals. As standard the same pure organic tin compound should be used rather than a salt such as $SnCl_2$.

7. *Determination of lithium in antidepressives.* Lithium carbonate, sulphate and aspartate are used for the treatment of manic depressive illnesses with a single dose of 30–50 mg lithium salt. Lithium can be determined after dilution at 670.7 nm or directly at the insensitive resonance line at 323.3 nm with flame AAS.

8. *Copper determination in herbs such as lupuli strobuli.* Lupuli strobuli is applied with baldrian tincture as a tranquilizer. The DAC (Deutsches Arzneimittel Codex) allows an upper limit of 400 ppm copper in this product which can easily be checked with AAS.

9. *Determination of silicon in dimethylpolysiloxane.* Dimethylpolysiloxane is used along with pancreatin and dihydrocholic acid for the treatment of gallbladder and digestive disorders. The compound is extracted with an organic solvent such as MIBK or chloroform and silicon determined in a N_2O–acetylene flame at the level of 5–20 μg/ml Si. A spectral slit-width of 2 A° is recommended as silicon has a large number of adjacent resonance lines that can cause curvature of the calibration plot on account of different absorption coefficients of individual resonance lines. Organic silicon compounds should be used as standards.

10. *Gold content of an organic gold compound used for the cure of chronic polyarthritis.* Aurothioglucose is a water-soluble compound with a labelled gold assay of 50% which is suspended in oils and applied for the treatment of polyarthritis. Gold is determined in an air–acetylene flame in the range 5–20 μ/ml after decomposition of the compound with aqua regia.

11. *Determination of iron in diverse iron-containing medicaments.*

12. *Determination of traces of lead in zinc oxide and zinc oxide formulations.* ZnO is used in powder, pastes, ointments and suppositories due to its light astringent properties. DAB 7 allows a limit of 20 ppm and *European Pharmacopoeia* 100 ppm lead in zinc oxide. Lead can be specifically determined in the ppm–ppb range in a large excess of zinc at 283.3 nm with flame or flameless AAS.

13. *Palladium in synthetic drugs.* The synthesis of various drugs requires a catalytic hydrogenation step using palladium. The determination of traces of palladium in drugs is therefore required. The palladium content can be evaluated in the ppm–ppb range using graphite tube or furnace flameless AAS, and deuterium background correction.

14. *Zirconium determination in material used in dental medicine.* Zr can be determined in the samples after sodium carbonate–borate chemical decomposition and extraction with HCl. A N_2O–acetylene flame is used. The addition of ammonium fluoride or hydrofluoric acid is essential to enhance the analytical sensitivity.

15. *Mercury in pharmaceuticals, plant materials and herbs.* The use of the cold vapour technique permits a very sensitive (ppb) determination of mercury. After chemical treatment, extraction, wet chemical decomposition, etc., the sample is treated with sodium borohydride and the mercury vapour is carried with nitrogen into a long quartz cell and measured at 253.6 nm. This technique allows a separation and enrichment of mercury traces from the sample matrix, with few if any interferences.

16. *Thallium, cadmium and lead in herbs.* After acid decomposition of the plant material in quartz Kjeldahl flasks or in a teflon apparatus in a closed system, Cd, Tl and Pb can be determined in the ppm–ppb range with flameless AAS in graphite tube/furnace.

17. *Potassium determination in urine: a bioavailability study of sustained-release potassium tablets.* Potassium can be determined in urine either after sufficient dilution at 766.5 nm or with little or no dilution directly at 404.7 nm.

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