2 ATOMIC ABSORPTION SPECTROMETRY

2.1 General

Atomic absorption spectrometry (AAS) uses as an analytical property the absorption of radiation by free atoms of the element. The loss of primary radiation is a measure of the concentration of free atoms of the element that absorbed the radiation. The differences in energies between the electronic states of an atom are characteristic of each element.

The transition of an atom from a lower energy level *m* to a higher energy level *n* is not spontaneous, but rather it is forced by the presence of radiation of a suitable frequency ν_{mn}. The energy of the photon $h\nu_{mn}$ must correspond to the difference in energy between the levels *m* and *n* according to equation (2.1)

$$\Delta E_{mn} = h\nu_{mn} = \frac{hc}{\lambda} \tag{2.1}$$

where *E* is energy, *h* is Planck's constant, *c* is the speed of light and λ is the wavelength of radiation. The absorption of the photon creates an excited atom, which can spontaneously switch to a lower energy state *m*, while the energy difference ΔE_{mn} can radiate in the form of a photon of the same frequency $\nu_{mn}=\nu_{nm}$. The difference of energies corresponding to the transition between the energy states *m* and *n* when the photon is absorbed or emitted is the same in absolute value and differs only in sign. This property of matter to emit and absorb electromagnetic radiation of the same wavelength is expressed by Kirchhoff's law, which is also the basis for the analytical use of atomic absorption.

For the formation of free atoms, a flame is most often used in AAS, which, depending on the type of fuel and oxidiser, reaches a temperature of 2000–3150 K. At these temperatures, most of the free atoms of most elements are in the ground energy state E_0. The electron transitions from the ground state, as well as the emission processes ending at this energy state, are called **resonant**. In AAS, the highest probability is for transitions from the ground state to the nearest excited state with energy E_1. These transitions generate **basic resonance lines**, which are the most sensitive and specific for the atoms of individual elements, as they are a function of the interaction of a positively charged nucleus and the electronic shell configuration of a given element.

The general two-level system *n-m* can then be replaced by the system 1-0, wherein the proportional representation of the number of atoms in the higher and lower energy is defined by **Boltzmann's distribution law**

$$\frac{N_1}{N_0} = \frac{g_1}{g_0} \cdot exp\left(-\frac{E_1-E_0}{kT}\right) \tag{2.2}$$

where N_0 and N_1 is the concentration of atoms in the ground and excited state, respectively, expressed by their number in volume unit [m^{-3}], g_0 and g_1 re statistical weights of these states, *k* is the Boltzmann constant [J K^{-1}] and *T* is absolute temperature [K].

The proportion of excited atoms N_1 (assuming $g_1=g_0$) is very small in the flames at the differences of energy levels E_1-E_0 corresponding to the emission of radiation in the visible and ultraviolet region of the spectrum (e.g. for ν=1015 Hz (λ=300 nm) and

T=3000 K is N_1/N_0=1,5.10^{-7}). Thus, the majority of free atoms are in the ground state and therefore are able to absorb the radiation of their resonant wavelengths.

Atomic absorption spectra are typically found in the wavelength range of 190-900 nm. If monochromatic radiation passes through a suitable **absorption medium** of thickness b and the number of free atoms in the ground state N_0, the radiation flux is attenuated from the original value Φ_0 [J s^{-1}] to the value Φ. The mathematical expression of these facts is **Bouguer-Lambert-Beer law** in the form:

$$A = log \frac{\Phi_0}{\Phi} = \kappa_\lambda \cdot b \cdot N_0 \tag{2.3}$$

where the **absorbance** A, defined as the logarithm of the ratio of the original and attenuated radiant flux, is proportional to the thickness of the absorbing layer b and number of free atoms in the ground state N_0. The proportionality constant κ_λ, the magnitude of which corresponds to the effective cross section of the atom for photon absorption, is characteristic of the absorbing element at a given wavelength and is called the atomic **absorption coefficient,** which has an area dimension [m^2]. At constant temperature, the constant number of electrons in the flame and stable conditions of sample nebulising into the flame, the number of atoms at the ground energy state is proportional to the concentration of the **monitored element (analyte)** in the solution.

In addition to absorbance, an important parameter is **transmittance τ (transmittance),** which is defined as the ratio of the radiant flux passed through the absorption medium Φ to the radiant flux entering into the medium Φ_0.

$$\tau = \frac{\Phi}{\Phi_0} \tag{2.4}$$

We use transmittance for setting the maximum of the resonant line wavelength in the spectral interval circumscribed by the monochromator. There is a simple conversion relationship between absorbance and transmittance:

$$A = -log\ \tau \tag{2.5}$$

The AAS method, like a number of analytical methods, is a comparative method and the measured quantity is absorbance. The value of absorbance as a measure of the concentration of the monitored element does not depend on the size of the radiant source, but the irradiance (radiant flux density) affects the smallest measurable absorbance (signal-to-noise ratio) and thus the detection limit. The results are evaluated using a calibration curve constructed by measuring the absorbances of calibration solutions of known concentration or by the method of standard addition.

In the analysed samples, the monitored element usually occurs together with other elements or compounds that can significantly affect the absorbance value. This influence of the sample matrix on measured signal is known as **interference**. We distinguish between spectral and non-spectral interferences.

Spectral interferences are caused by imperfect resolution of the absorption signals of analyte and interferent. Overlapping of absorption lines in atomic spectra is quite rare in AAS. However, the radiation of the primary source can be attenuated not only by free analyte atoms, but also by **non-specific absorption**, which is caused by **scattering of radiation** on non-evaporated aerosol particles and **molecular absorption**. While

scattering is most pronounced in the short wavelength region (up to 250 nm), absorption by molecules has broadband character and occurs throughout the whole spectral range. Non-specific absorption is additive to the analyte signal and always causes a **positive error**. The correction of non-selective absorption is most often performed by means of a source of continuous radiation, which is a deuterium lamp in the ultraviolet region (up to 350 nm) and a halogen lamp with a tungsten filament in the visible region.

Non-spectral interferences include other phenomena, e.g. **interfering effect of sample transport** into the flame (changes in flow rate and nebulising efficiency due to different surface tension, density and viscosity of samples), **interfering effect of condensed phase evaporation** due to compounds with different volatility (signal decreasing effect of phosphates in calcium determination or signal increasing effect of fluorides in the determination of aluminium) and **interference in the gas phase,** due to a shift in the dissociation and ionisation equilibria and changes in the spatial distribution of free atoms in the flame. **Non-spectral interferences are not additive, so** they can cause both positive and negative errors, and they can accidentally eliminate each other. The composition of the calibration solutions should therefore correspond as closely as possible to the **matrix of the sample** in which analyte is monitored. If the absorbance of the analyte in the sample is then measured under the same conditions (nebulisation, wavelength, flame composition, burner slit length, etc.), the relationship (2.3) is simplified to the direct relationship between the absorbance and the analyte concentration in the sample

$$A = k \cdot \rho \tag{2.6}$$

where ρ is the mass concentration of the analyte. However, the linear dependence is valid only for low analyte concentrations and the dependence becomes non-linear at higher concentrations.

The slope of the calibration curve $k=dA/d\rho$ determines the sensitivity of the method. AAS often states the **characteristic concentration of the element**, which is the concentration that absorbs 1% of the original radiation (τ =0.99) and corresponds to the absorbance value of A=0.0044. The characteristic concentration is expressed in the mass concentration of metal in the solution without impurities and differs for individual elements by up to several orders of magnitude.

The AAS method with flame ionisation makes it possible to measure the concentration of about 60 elements (metals and transition elements) and is used in the analysis of samples of various origins. The importance of this method lies in monitoring low contents of toxic elements in environmental samples, where it is very well applied due to its high sensitivity and selectivity.

2.2 Instrumentation

The basic components of each atomic absorption spectrometer, as arranged in the optical axis, are:

- **source of monochromatic resonant radiation** of the monitored element
- **absorption medium** with free atoms
- **monochromator** for isolation of the resonant line of primary radiation
- **a radiation detector** that converts photons (radiant flux) into an electric current

2.2.1 Radiation source

The source of primary radiation is a low-pressure, neon-filled **hollow cathode lamp** (HCL). The lamp (Fig. 2.1) operates in glow-discharge mode at a current of several milliamperes and a pressure of approx. 0.1 kPa. **It emits the line spectrum of the element** from which the hollow cathode is made of or which is contained in the hollow cathode material. This imparts the high selectivity of this method, which makes it possible to determine the concentrations of individual elements in a sample, usually without the need for preliminary separation. Excitation conditions at both low pressure and temperature guarantee that the resonant radiation is highly monochromatic and has a **spectral line half-width** (i.e. a width measured at half the line profile height) of only about 0.001 nm.

Fig. 2.1: Hollow cathode lamp

The radiation emitted by the lamp is modulated. Modulation is performed electrically or mechanically by a chopper. Modulation makes it possible to measure only the radiation of the lamp, not the radiation emitted from the atomising medium, e.g. from the flame. The detector's alternate current signal amplifier is also tuned to the same modulation frequency. A stabilised current source with a voltage of about 400 V is used to supply the lamp. At present, hollow cathode lamps are produced for more than 60 elements. For elements that have similar physical properties and are similarly cathodically sputtered, it is possible to produce lamps with a multi-element cathode. In multi-element lamps, the cathode is made by sintering a mixture of two to six powdered metals.

2.2.2 Atomiser

The absorption medium, where the free atoms of the analyte are formed, must have a temperature of at least 2000–3000 K. The simplest medium for atomisation is a **laminar premixed flame**, which is obtained by laminar combustion of a premixed mixture of acetylene with air or nitrous oxide in a special burner. The burner orifice has the shape of a narrow slit 10 cm long for an acetylene-air flame and only 5 cm long for an acetylene-nitrous oxide flame because of the higher burning rate. The length of the slit also determines the maximum achievable thickness of the absorption medium. The analysed sample is introduced into the flame in the form of an aerosol. The nebulisation of the solution is carried out pneumatically in a nebuliser by means of the pressure of the oxidising gas, which is air or nitrous oxide. Each atomic absorption spectrometer is equipped with regulation and measurement of the flow of the fuel and oxidiser. Setting the ratio of the two gases in the mixture provides either an oxidative or reductive flame type. The reducing flame is suitable for atomising elements that form thermostable oxides (e.g. Cr, Al). The composition and temperature of the flame changes with height. Thus, for each element, there is an optimal zone in the flame given the height above the slit of the burner, where the concentration of free atoms is highest. This height must be determined experimentally. The position of the burner is therefore adjustable in the vertical and horizontal directions.

2.2.3 Monochromator

The **grating monochromator** is placed behind the flame. It serves to isolate radiation of a suitable wavelength. By rotating the grating, the wavelength of the resonant line is set to the maximum transmittance. A common monochromator has input and output slits with an adjustable **spectral range width** from 0.1 nm to 2.0 nm. As already mentioned, the width of the resonance line profile is approx. 0.001 nm. The width of the spectral range is chosen so that the **ballast radiation** (radiation that cannot be absorbed by the analyte) of lines close to resonant radiation wavelengths does not reach the detector. This would cause curvature of the absorbance dependence on concentration (invalidity of the Bouguer-Lambert-Beer law). If the resonance line is alone in the spectrum of the lamp, the instrument can operate with a wider slit (e.g. 0.5-1.0 nm).

2.2.4 Detector

The **photomultiplier** with a photocathode whose sensitivity is sufficient for the observed spectral range (i.e. from 190 to 900 nm) is placed close behind the exit slit of the monochromator for detection of the radiation flux Φ_0 and Φ (Eq. (2.4)). The obtained photocurrent is amplified by the voltage applied to the dynodes of the electron multiplier and also by another amplifier. AAS uses phase-sensitive amplifiers (lock-in) tuned to the frequency of the modulating radiation of the lamp. The system is equipped with a logarithmic transducer for direct reading of absorbance values on a linear scale.

2.3 Working with GBC SensAA Dual spectrometers

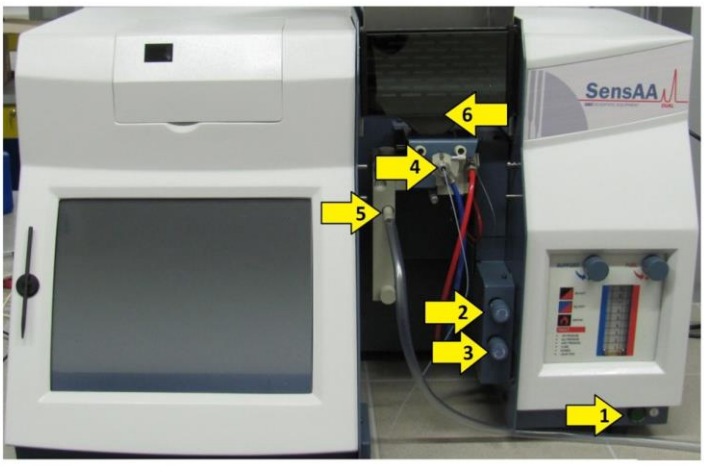
2.3.1 Description of spectrometers

To measure the atomic absorption in this laboratory task, a two-beam spectrometer (GBC, model Sens AA Dual), equipped with deuterium lamp for the correction of non-selective absorption was used. The radiation source is a hollow cathode lamp, the radiant flux of which is modulated electrically by interrupting the supply current with a precisely set frequency. With this frequency, the amplifier is electronically synchronised. The instrument is equipped with a grid monochromator of the Fastie-Ebert construction including a grid with 1800 grooves/mm and a focal distance of 333 mm. The width of monochromator slit is in the range of 0.2-2 nm. For detection of the radiant flux relative intensities, the instrument is equipped with a universal photomultiplier with a working range of 175-900 nm.

The GBC spectrometer (type Sens AA) has one position for a hollow cathode lamp, powered by modulated direct current of 3-20 mA according to the measured element and the manufacturer's recommendations. It is necessary to let the HCL glow for 15-20 minutes before the start of the analysis. The lamp in the working position can be adjusted in the vertical and horizontal directions so that as much of the primary radiation as possible reaches the detector.

!!! ATTENTION !!! It is forbidden to manipulate the screws of the deuterium lamp!

The location of the control elements of the spectrometer can be seen in Fig. 2.2 and 2.3.

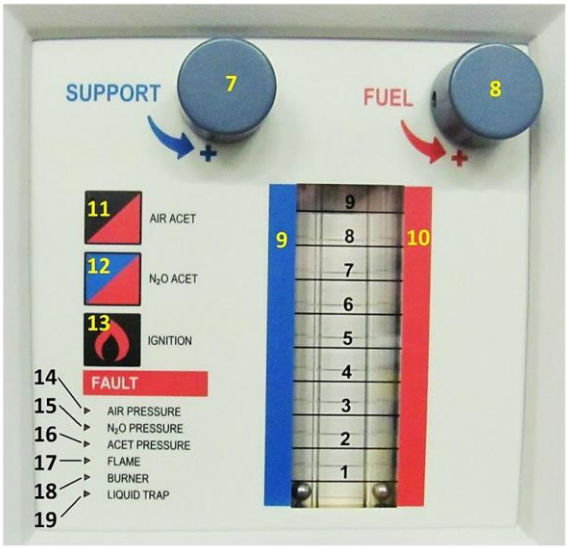


1. Power switch
2. IN-OUT: adjustment of the burner position in the horizontal direction
3. UP-DOWN: adjustment of the burner position in the vertical direction
4. Adjustable nebulizer with capillary for sample aspiration
5. Drain the condensed part of the aspirated sample
6. Burner with slit

Fig. 2.2: Atomic absorption spectrometer GBC model SensAA

Besides to setting the gas flows, the device is fully computer controlled via SavantAA software. The inlet pressure of the fuel and oxidizer, automatic ignition of the flame, the flame type selection with automatic checking the safety composition of mixture acetylene-nitrous oxide, and the correct choice of the burner are a microprocessor controlled in the gas module. In the event of any fault in this section, the flame is automatically extinguished and cannot be ignited until the fault has been eliminated. For the acetylene-nitrous oxide flame, it is absolutely necessary to use a burner with a slit of only 50 mm (marked N_2O-ACET). Acetylene-air flame can be operated in both types of burners. The use of the correct burner is ensured by the switching connector. With a wrong burner for the high-temperature acetylene-nitrous oxide flame, this mixture cannot be ignited.

Measurements of absorbance or concentration of prepared calibration solutions and samples, and the calibration curve can be viewed on the screen and printed.



7. SUPPORT: oxidizer flow control
8. FUEL: fuel flow control
9. Flowmeter for oxidizer
10. Flowmeter for acetylene
11. AIR ACET: button for starting and setting the flow of gases for the acetylene-air flame. It serves also for extinguishong the flame.
12. N2O ACET: button for starting and setting the flow of gases for the acetylene-nitrous oxide flame. It serves also for extinguishong the flame.
13. IGNITION: flame ingnition button
14. Indication of incorrect air pressure
15. Indication of incorrect nitrous oxide pressure
16. Indication of incorrect acetylene pressure
17. Indication of flame failure
18. Indication of the use of the wrong type of burner or incorrect insertion of the connector
19. Signaling of the empty siphon of the drain bottle

Fig. 2.3: Control panel of the GBC SensAA spectrometer with automatic flame control

2.3.2 Putting the instrument into operation

The atomic absorption spectrometer is switched on with the green switch (marked 1 in Fig. 2.2). When the power switch is turned on, the Windows 7 operating system and the GBC SavantAA control software start automatically. At the end of the day, the computer and the device are switched off together using the green switch. The GBC SavantAA software (Fig. 2.4) enables instrument control using the system menu, buttons in the horizontal toolbar and module buttons in a vertical panel.

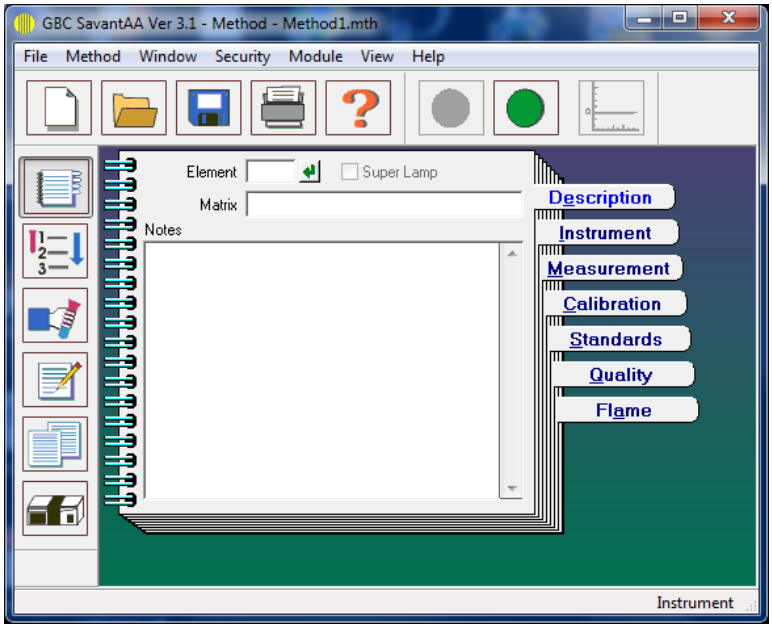


Fig. 2.4: Control panel of the software: module Method

The function of the horizontally (Tab. 2.1) and vertically (Tab 2.2) positioned buttons is described in the following tables.

Table 2.1: Functions of the vertically positioned buttons

New		Create a new file
Open		Upload a file
Save		Save a file. When saving a newly created file, the software automatically chooses a name for that file. When working in laboratories, please use the File-Save as command and save the methods or results in the form *SURNAME-ANALYTE.MTH* or *SURNAME-ANALYTE.RES, respectively,* into the Student Laboratory folder on the D: disc**.** *Attention: the type of processed files is determined by selecting the vertically located button or by selecting the Module menu.*
Print		Print the entire content of the module
Help		Help
Stop		Stop the measurement sequence
Start		Start the measurement sequence
Zero		Setting the absorbance to zero (blank)

Table 2.2: Functions of the horizontally positioned buttons (selection of module)

Method		Editing of measurement conditions
Samples		It is used to setting the type (1st column of the table) and name (2nd column of the table) of the measured samples. The number of rows in the table is changed using the *Insert* and *Delete* keys or the buttons at the bottom of the panel (the function of the buttons is obvious from their graphic form). The sample name (*Label*) is written directly from the keyboard. The sample type is selected (*Measurement*) by right-clicking on the required line and selecting *Properties* from the menu. The required sample type is selected in the *Measurement Type* and the selection is confirmed by clicking on the OK button. Important sample types are *Sample* and *Calibration*. Note: The *Calibration* sample type includes the entire calibration curve. Therefore, individual calibration solutions do not have to be entered
Analysis		From this module, the *Sequence* panel is important, in which the order of the measured samples is selected. In the *First Measurement* line, select the sequence number of the sample corresponding to the sample table (see above) that will be measured first. Additional samples are measured in the order in which they are listed in the table. If *Measure* to *End* is checked, the samples are measured up to the last recorded sample; otherwise, the last measured sample will be the sample with the sequence number listed in the *Last Measurement* line
Results		table of measurement results
Report		setting the parameters of the output
Instruments		optimization of instrument parameters Note: the lamp position optimization module is activated by right-clicking on the middle upper part of the device diagram (Fig. 2.5)

Ignition of the flame

The air compressor is started first. The main valve of the acetylene cylinder is opened by turning it by 1.5 turns (not to the maximum position) and the side valve is also opened **in the presence of the assistant.** The output pressure of acetylene is set to 60–70 kPa and the air compressor is pressurized. The pressing a button (11, Fig. 2.3) opens the gas supply and button (13) ignites the flame. As soon as the flame burns, check the setting of the air flow through the rotameter (9) (approx. 5 units of the rotameter) and the acetylene flow through the rotameter (10, Fig. 2.3) (1–2 units). Immediately after ignition, distilled water begins to nebulised into the flame. Evaporation of the water in the nebulizer chamber leads to its cooling and thus protects the inner Teflon coating. If the flame lit, a solution (sample or distilled water) must always be nebulised into the flame.

During combustion, the device monitors dangerous states that may occur during operation and indicates such a situation by series of diodes (14-19, Fig. 2.3). If any error is detected, the flame goes out immediately. Extinguishing the flame after the end of

the measurement is achieved by pressing the button (11, Fig. 2.3). Do not let the flame burn unnecessarily and switch it off immediately after the measurement of the prepared series of samples.

Measurement

Under the optimised conditions, the sample can be measured according to the prepared method (*Method* module) and sequence of samples (*Samples* and *Analysis* modules). The measurement is activated by pressing the *Start* button or by using the menu command *File-Start Analysis*.

Attention: if the measurement is started, the zero absorbance is automatically set again, therefore it is necessary to nebulise only distilled water at this moment and not the solution containing the determined element.

The actual measurement of the absorbance of a given sample is activated by pressing the *OK* button in the *Manual sampling* window, which opens automatically after starting the measurement. It is possible to measure last sample again by pressing the *Repeat Sample*. In the *Results* module, the measurement results can be monitored continuously. Premature termination of the measurement is performed by pressing the *Stop* button; otherwise, the measurement will end itself after measuring all samples entered in the *Samples* module.

2.4 Instructions for laboratory tasks

The aim of the laboratory task is the determination of chromium and copper or calcium and magnesium by atomic absorption spectrometry. The task includes the following parts:

a) Prepare calibration solutions of Cr and Cu or Ca and Mg.
b) Familiarise yourself with the instrument and develop methods for the determination of Cr and Cu or Mg and Ca.
c) Optimise the measurement conditions.
d) Measure the calibration curve and the samples by the developed method.
e) Evaluate the mass concentrations of the elements in the samples and write a protocol.

2.4.1 Preparation of calibration solutions

- 100 mL of the **Cr** stock solution with a mass concentration of 1 g/L Cr is prepared by weighing the calculated amount of K_2CrO_4 and dissolving it in distilled water.
- 500 mL of the **Cu** stock solution with a mass concentration of 1 g/L is prepared by weighing the calculated amount of $CuSO_4 \cdot 5\ H_2O$ (purity p.a.) and dissolving it in distilled water.

- 500 mL of the **Ca** stock solution with a mass concentration of 1 g/L Ca is prepared by weighing the calculated amount of $CaCO_3$ (purity p.a.) and dissolving it in 10 mL of diluted HCl (1:1, v/v).
- 500 mL of the **Mg** stock solution with a mass concentration of 0.1 g/L Mg is prepared by weighing the calculated amount of $MgSO_4 \cdot 7\ H_2O$ (purity p.a.) and dissolving it in distilled water.

All these stock solutions are too concentrated, so they must be diluted ten-fold (25 mL pipetted into a 250 mL volumetric flask for Cr, Cu, and Ca) or twenty-fold (25 mL pipetted into a 500 mL volumetric flask for Mg) before preparing the calibration solutions. These solutions have a concentration of 0.1 g/L Cr, 0.1 g/L Cu, 0.1 g/L Ca, and 0.005 g/L Mg. From these dilute solutions, prepare the final calibration solutions:

- for the determination of Cr: 0, 2, 4, 6, 8 and 10 mg/L Cr
- for the determination of Cu: 0, 2, 4, 6, 8 and 10 mg/L Cu
- for the determination of Ca: 0, 2, 4, 6, 8 and 10 mg/L Ca
- for the determination of Mg: 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/L Mg

Calibration solutions for Ca and Mg or Cu and Cr are prepared in a mixture. Prepare 100 mL of each calibration solution. The calculated volume of the diluted stock solution of the given metal is measured by a burette. **Before filling the volumetric flask with water, the mixture of Cu and Cr is acidified by adding 1 mL of concentrated HNO_3.**

2.4.2 Creating a method for the determination of Cr, Cu, Ca or Mg

Creating a new measuring method or editing an existing method is performed in the *Method* module, which contains several items (Table 2.3). The individual method parameters are set in accordance with Table 2.4 and other instructions of the assistant.

Table 2.3: Method module

Description	Choose the analyte. Other parameters have the character of a label and are not mandatory.
Instrument	The values of lamp current (*Lamp Current*), wavelength (*Wavelength*), slit width (*Slit Width*), slit height (*Slit Height*) and (if needed) non-specific absorption correction (*Background Correction*) are set.
Measurement	Select the measurement mode (*Measurement Mode*) and write the reading time of one replica (*Read Time*), the detector time constant (*Time Constant*) and number of replicates (*Replicates*)
Calibration	Select the type of calibration curve (*Calibration Mode*), units (*Conc. Units*), number of decimal places (*Conc. Decimal Places*), check the zero before calibration (*Zero Before Calibration*) and in case of optimisation, also check zero between samples (*Zero between samples*)

Table 2.3: continuing

Standards	Expand the table to the appropriate number of rows (use the buttons at the bottom of the page) and record the concentrations of the calibration solutions. *Attention: using the last button on the right, you can graphically display the calibration curve after measuring the calibration solutions*
Quality	Quality management; this item is not used in this laboratory task.
Flame	GBC SensAA settings cannot be controlled directly from the computer, so parameters may not be filled in. *Attention: pressing the Optimise button on this page opens a window in which it is possible to monitor the absorbance values in real time. The flame composition will be optimised manually, so do not press the buttons in this window.*

Table 2.4: Data for creating a measuring method

Element	copper	chromium	magnesium	calcium
Description				
Element	Cu	Cr	Mg	Ca
Instrument				
Lamp Current	8 mA	8 mA	5 mA	5 mA
Wavelength	327.4 nm	425.4 nm	285.2 nm	422.7 nm
Slit Width	0.5 nm	0.5 nm	0.5 nm	0.5 nm
Slit Height	normal	normal	normal	normal
Background Correction	no	no	yes	no
Measurement				
Measurement Mode	Integration	Integration	Integration	Integration
Read Time	3.0 s	3.0 s	3.0 s	3.0 s
Time Constant	0.4 ms	0.4 ms	0.4 ms	0.4 ms
Replicates	5	5	5	5
Calibration				
Calibration Mode	Conc. Least Square	Conc. Least Square	Conc. Least Square	Conc. Least Square
Conc. Units	µg/mL	µg/mL	µg/mL	µg/mL
Conc. Decimal Places	2	2	3	2
Zero Before Calibration	yes	yes	yes	yes
Zero Between Samples	yes/no*	yes/no *	yes/no *	yes/no *

* for optimising the burner position and flame composition **yes**, for measuring calibration curve and samples **no**

2.4.3 Optimisation

HCL position setting

In the *Instrument* module (Fig. 2.5), right-click in the upper left part of the instrument scheme and select *Optimise: HC/DC*. Two indicators of emitted radiation flux appear on the computer screen. The left indicator shows the radiation flux of HCL, the right indicator shows the radiation flux of the background corrector (deuterium lamp) (Fig. 2.6).

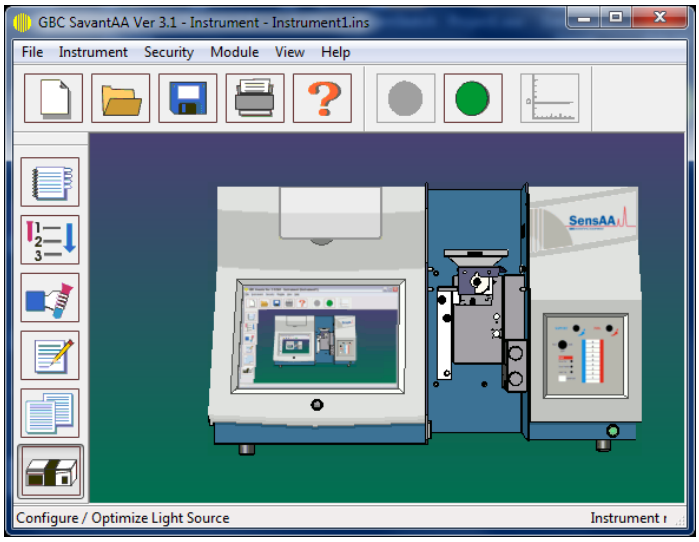


Fig. 2.5: Control panel: Instrument module

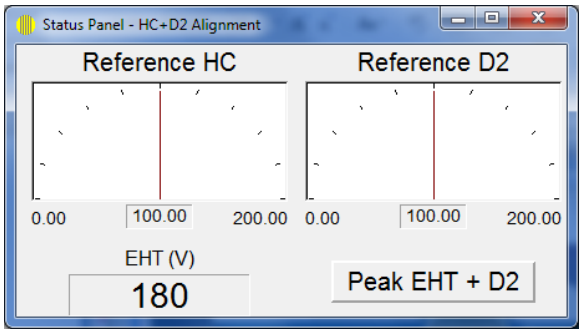


Fig. 2.6: Status panel of lamp alignment

Using the screws located on the left side of the instrument, the HCL is adjusted to the position at which the left indicator reaches maximum. DO NOT set the position of the background corrector! When the adjustment is complete, press the *Peak EHT+D2* button to recalibrate the voltage on the photomultiplier (EHT). If the voltage changes significantly, repeat the whole procedure.

Adjusting the horizontal position of the burner

Before igniting the flame, adjust the horizontal position of the burner using the screw (2). The image of the red beam emitted by the HCL is observed on a strip of white paper and the beam should pass exactly over the burner slit along its entire length.

Adjusting the vertical position of the burner

After optimisation of HCL horizontal position, the flame ignites. A reducing flame (slightly luminous) is set for Cr, a stoichiometric composition of the flame in the case of Ca and Mg and an oxidising flame (low fuel) in the case of Cu. The composition of the flame is regulated only by the screw (8) (*FUEL*). Immediately after ignition, distilled water begins to be nebulised into the flame. The burner is marked on the right side with a scale of 0-8; start at position 0. In the *Samples* module, edit the table so that it contains ten rows and enter the *Sample* type in each row. The sample names will correspond to the position of the burner (increment of 0.5). Start the measurement with the *Start* button. During the entire measurement, nebulise the same solution, usually the one with the highest concentration. Before each further measurement, move the burner by half a mark. The position that corresponds to the maximum measured absorbance is the optimal position, and all other measurements will be made in this position. The measured absorbance values are plotted on a chart.

Setting the flame composition

The optimal flame composition is set in a similar way as the vertical position. The flame composition is especially critical for the determination of Cr. The composition of the flame is changed by setting acetylene flow from mark 1 to mark 4 (very luminous reducing flame with excess fuel) on the rotameter (8) with increments of 0.5. In the *Samples* module, edit the table to contain at least ten rows, and enter a *Sample* type in each row. Names of samples should correspond to the flow of acetylene. Start the measurement with the *Start* button. During the whole measurement, the same calibration solution is nebulised as in the case of adjusting the vertical position of the burner. The acetylene flow that corresponds to the maximum measured absorbance is the optimal flow, and all other measurements will be made at this flow. The measured absorbance values are plotted on a chart.

After completing the optimisation of the flame composition, it is suitable to measure unknown samples and compare them with the measured standard. If the absorbance of the sample is higher than that of the most concentrated calibration solution, it must be diluted before measuring the calibration curve and samples.

2.4.4 Measurement of calibration curve and samples

Before the measurement, fill the unknown samples in the volumetric flask to the mark with water and acidify with 1 mL of concentrated HNO_3 in the case of a mixture of Cr and Cu. Shake the flasks thoroughly and dilute samples if necessary (see

above). The table in the *Samples* module is prepared for the measurement so that it contains at least seven rows. The first sample (first row) will be the *Calibration* type, and the others will be the *Sample* type. For these samples, write a name corresponding the sample label on the flask. It is also advisable to make a note as to whether the samples have been diluted by adding in the dilution factor.

After activating the measurement with the *Start* button, the calibration standard without analyte (Blank) is measured first followed by the other calibration solutions and the unknown samples. Each sample is measured three times; this is done by measuring samples A and B alternately.

Enough time must be allowed for to reach a steady-state between the beginning of solution nebulisation and the activation of the absorbance measurement; a period of five seconds is sufficient. If a sample absorbance/concentration is outside the calibration range and the student does not have a diluted sample, the entire calibration curve must be measured again after additional sample dilution.

Average absorbance values (Abs), values of relative standard deviations calculated from individual measurements (RSD) and the value of the concentration obtained from the calibration curve (Conc.) are recorded in the *Results* module.

2.5 Instructions for drawing up a protocol

The result of the measurement is the mass concentration (mg/L) of the elements in the given samples. If the sample was diluted before measurement, a recalculation must be performed.

In addition to the principles of AAS measurement, the protocol must contain tables and charts demonstrating the results of signal optimisation, the measurement of calibration curves and unknown samples. Do not forget to include the sample labels. The protocol also includes the procedure of calibration solution preparation, including the calculation of the weights of the relevant chemicals.

The report must also state the optimal conditions under which the absorption measurement was performed, i.e. wavelength, spectral interval width, lamp current, burner position, gas flows and voltage on the photomultiplier. Report the resulting mass concentration as the average of the measurements with a 95% confidence interval.

2.6 Control questions

1. What is the physical principle of AAS?
2. What does Kirchhoff's law express?
3. Which electron transitions are called resonant?
4. What do the basic resonance lines correspond to?
5. How are absorbance and transmittance defined and what is the relationship between them?
6. What is the atomic absorption coefficient and what does it depend on?

7. What does the Bouguer-Lambert-Beer law express?
8. Which optical quantities do we monitor in AAS and for what is their analytical purpose?
9. What does the term ‘characteristic concentration’ mean?
10. Which region of the wavelength spectrum does AAS use?
11. List the basic components of an AA spectrometer for flame atomisation.
12. What is the source of primary radiation in AAS?
13. Which instrumental factors affect the sensitivity and characteristic concentration in flame AAS?
14. What calibration methods can be used to evaluate the concentration of an element in a sample?
15. What do the accuracy and precision of analyte determination depend on?
16. What are the main analytical applications of AAS?
17. How is the absorption medium most often created in AAS and what temperatures must be reached in it?
18. What is used to isolate resonant lines and what is the function of this device?
19. What is a detector in AAS and what is its principle?
20. How is Boltzmann’s law formulated by the relative representation of atoms in lower and higher energy states?