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Interreg-IPA Cross-border Cooperation Programme Romania-Serbia

Academic Environmental Protection Studies on surface water quality in significant cross-border nature reservations Djerdap / Iron Gate national park and Carska Bara special nature reserve, with population awareness raising workshops

= RORS-462 =

FUNDAMENTALS OF ATOMIC AND MOLECULAR ABSORPTION SPECTROMETRY.

Instrumentation and Techniques of Atomic Absorption & UV VIS Spectrometry.

AEPS
common history, common future

Trainers

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www.aeps.upt.ro

25 - 26 November 2020

Interreg – IPA CBC
Romania - Serbia

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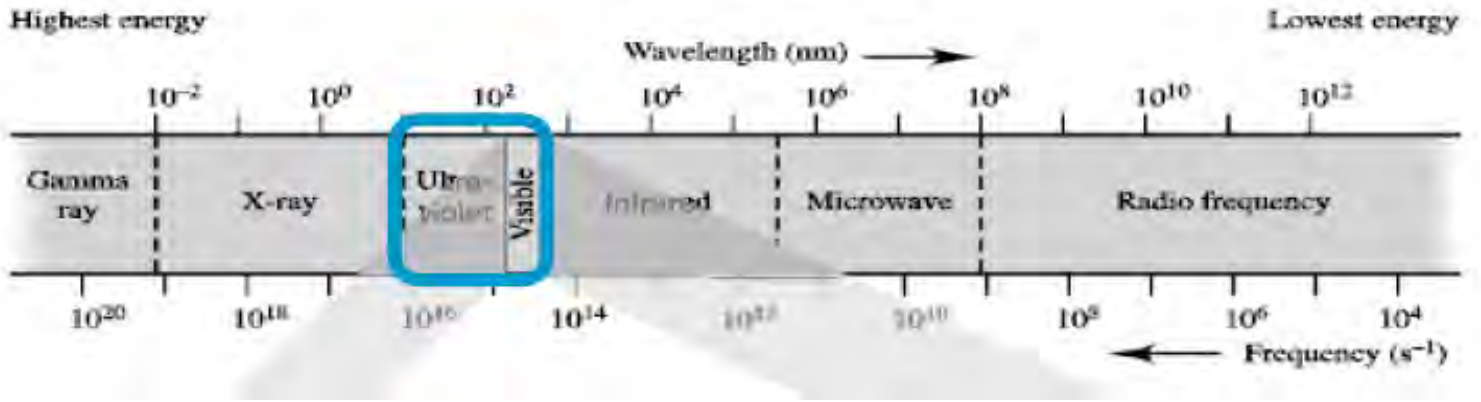
Fundamentals
Instrumentation and Techniques of UV VIS Spectroscopy

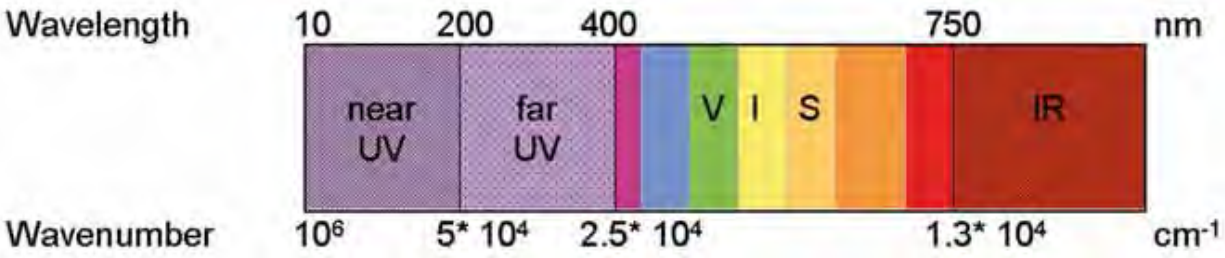
Application on Analytik Jena SPECORD 250 PLUS

Theory UV-VIS

Light absorbance and spectrum

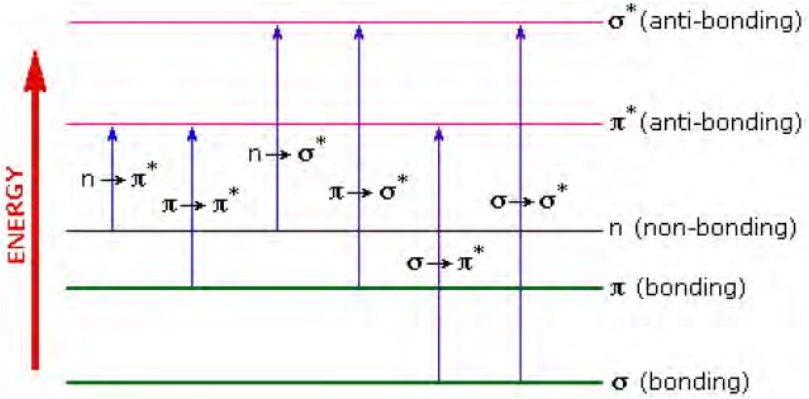
The interaction of electromagnetic radiation with solids, liquids or gases produces various effects, such as absorbance, reflectance or scattering. UV VIS spectroscopy exclusively investigates the interaction of radiation with matter in the ultraviolet and visible range.

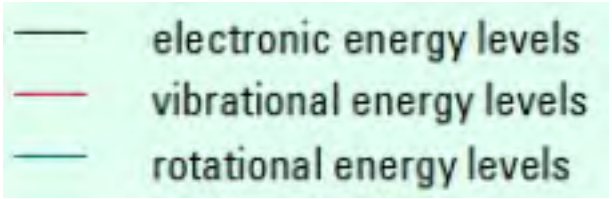


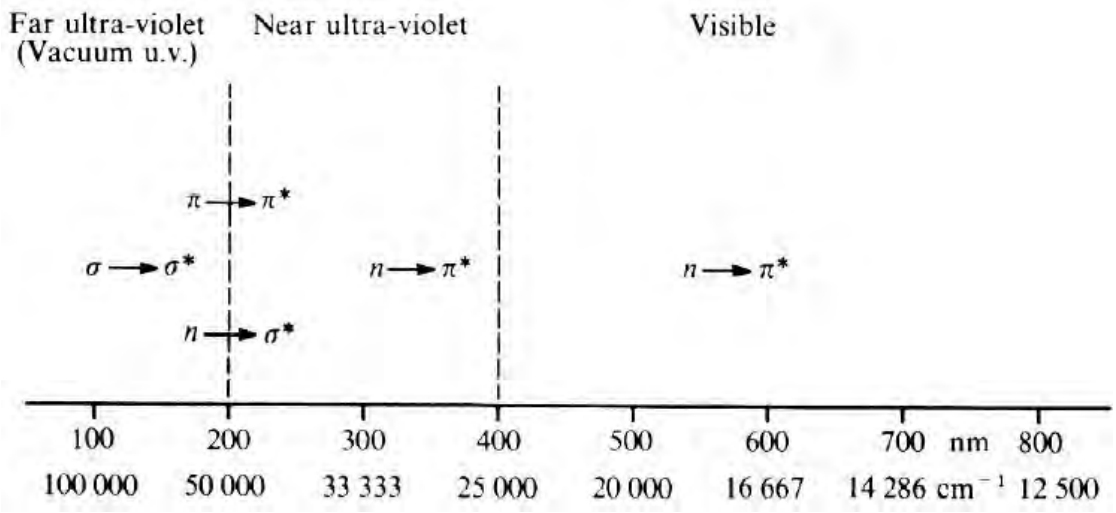


When atoms or molecules absorb electromagnetic radiation they are transformed from a ground state into an energetically excited state. Energy of a specific wavelength is absorbed in this process. The various molecular states have a relatively broad energy range in comparison with atoms.

Rotation and vibration of a molecule can be stimulated in the infrared range. The absorbance of defined packets of energy (quanta) by the valence electrons is observed in the range of visible and ultraviolet light.

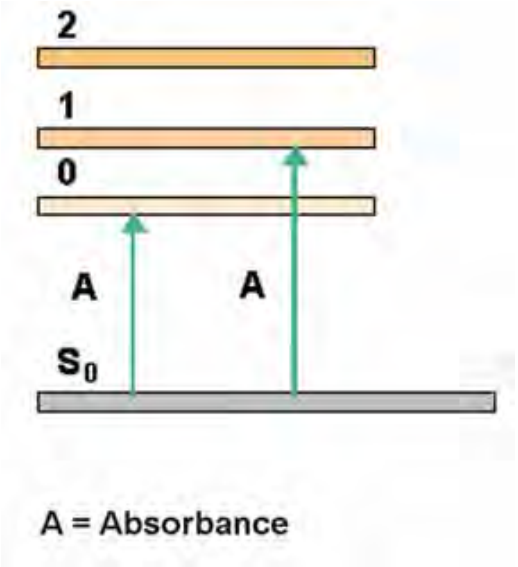


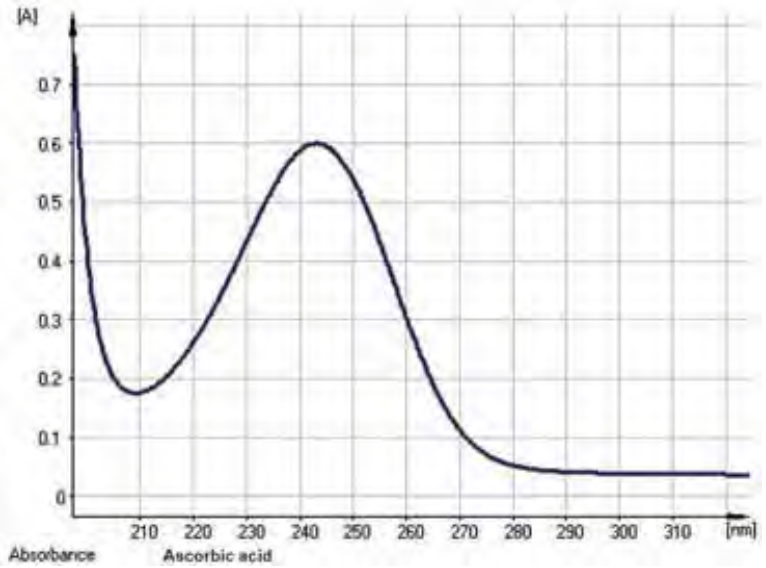




The energy of these quanta can be specified as the wavelength of the radiation.

The shorter the wavelength, the greater the energy of the quanta. The location of the absorbance points and the relative magnitudes of absorbance can be determined with UV VIS spectrophotometers.



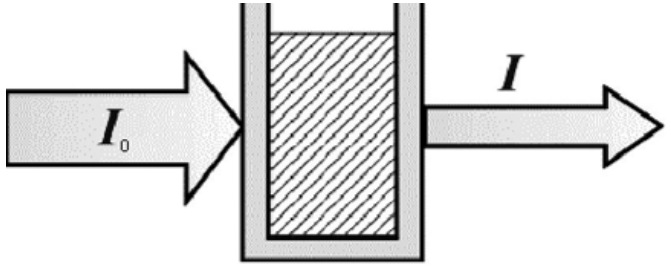


Absorbance and absorbance spectrum (ascorbic acid)

The energy of these quanta can be specified as the wavelength of the radiation. The shorter the wavelength, the greater the energy of the quanta. The location of the absorbance points and the relative magnitudes of absorbance can be determined with UV VIS spectrophotometers.

Transmittance and absorbance

If a light beam of intensity I_0 penetrates a medium of thickness d, the light beam is attenuated by the absorbance properties of the sample apart from reflectance and scattering losses. The exiting light beam (transmittance) now has the intensity I.



Transmittance

Transmittance T is defined by the following equation:

$$T = \frac{I}{I_0} \quad \text{or} \quad \%T = \frac{I}{I_0} \cdot 100\%$$

The following equation described the absorbance A of a sample:

$$A = -\log_{10} T = \lg\left(\frac{I_0}{I}\right)$$

In contrast to the transmittance, the absorbance of a solution therefore increases with increasing attenuation of the light beam.

Qualitative analysis

UV VIS spectra generally show relatively broad absorbance's bands from molecules. Compared with IR spectroscopy, in which many narrow bands are produced, the qualitative information yield is relatively low. Absorbance of organic molecules in the UV VIS range is often caused by chromophoric groups (color bearing species). The table below, provides an overview of chromophoric groups with their respective absorbance maxima.

The chemical group most strongly influencing molecular absorption characteristics is called a chromophore. Chromophores which can be detected by UV/Vis spectrophotometers always involve a multiple bond (such as C=C, C=O or C≡N) and may be conjugated with other groups to form complex chromophores.

Chromophore	Formula	Example	Absorbance maximum
carbonyl- (ketones)	RR'C=O	acetone	271 nm
carbonyl- (aldehyde)	RHC=O	acetaldehyde	293 nm
carboxyl-	RCOOH	acetic acid	204 nm
amido-	$RCONH_2$	acetamide	208 nm
azo-	-N=N-	diazomethane	339 nm
nitro-	$-NO_2$	nitromethane	280 nm

UV-VIS spectrophotometry can deliver the following qualitative information:

- ✓ identification of pure substances
- ✓ Identification of substances following HPLC separation (preferably with diode array
- ✓ systems)
- ✓ Purity testing (for example of proteins or DNA/RNA)
- ✓ Melting point curves of proteins and nucleic acids
- ✓ Differentiation of saturated and unsaturated compounds
- ✓ Differentiation of keto and enol forms
- ✓ Identification of carbonyl bands
- ✓ Clarification of bonding relationships and substituent effects
- ✓ Enzyme activities

Quantitative analyses

The **Bouguer-Lambert-Beer law** describes the relationship between absorbance and concentration

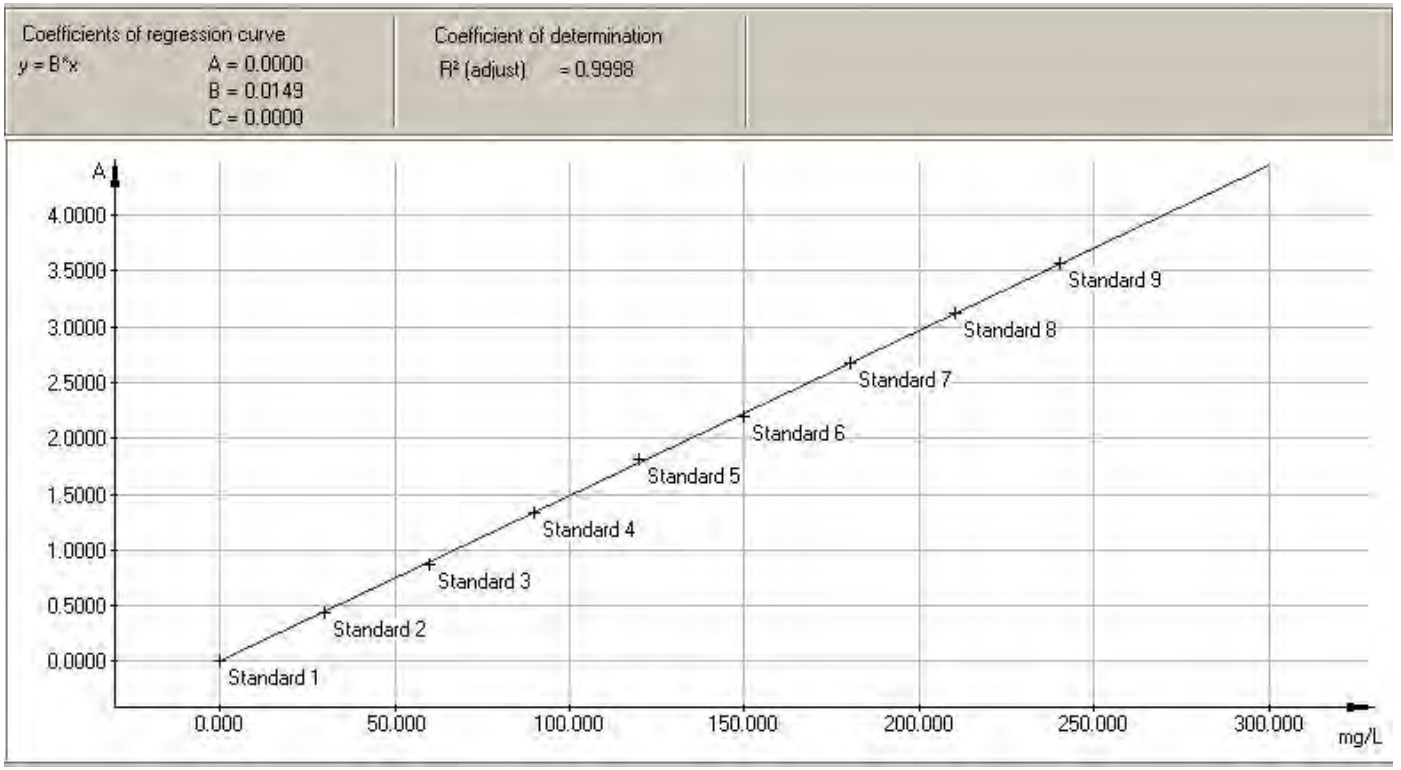
Bouguer (1696 – 1758) Absorbance is proportional to path length
Lambert (1728 – 1777)
Beer (1825 – 1863) Absorbance is proportional to molar concentration

$$A(\lambda) = \varepsilon(\lambda) \cdot c \cdot d$$

A (λ) - absorbance at wavelength λ
ε (λ) - molar logarithmic absorbance coefficient at wavelength λ (l/mol · cm)
c - concentration (mol/l)
d - path length of the cell in cm

The calibration process

Calibration means measuring samples with known concentration (standards) and the measured absorbance values are entered against the respective concentrations.
The Lambert-Beer law applies within the linear range of the calibration curve.
Non-linear calibration curves can also be used to a certain extent with the use of extended calculation models.



Sample preparation for photometric measurements

In most cases, the species under investigation have to be converted to colored compounds. The reagents for this color formation process are:

- ✓ Inorganic in nature. These reagents are not very widely distributed however.
- ✓ Organic in nature. More than 7500 such organic reagents are known. The colors observed result from interligand bands and charge transfer complexes.

Examples of parameters

aluminum	bromide	chloride	fluoride	manganese	nitrite	oxygen	sulfite
ammonia	BSB	chromate	gold	molybdenum	ozone	silver	surfactants (anionic)
ammonium	cadmium	CSB	iodine	sodium	phenol	nitrogen (total)	TOC
lead	chlorine	cyanide	potassium	nickel	phosphate	sulfate	peroxide
boron	chlorine dioxide	iron	copper	nitrate	residual hardness	sulfide	zinc

The absorption spectrum of a compound is one of its most useful physical characteristics, both as a means of **identification (qualitative analysis)** and of **estimation (quantitative analysis)**. If there is absorption in the visible and that absorption occurs in the red then the substance will be seen as green/blue since red and green/blue are complementary
colours

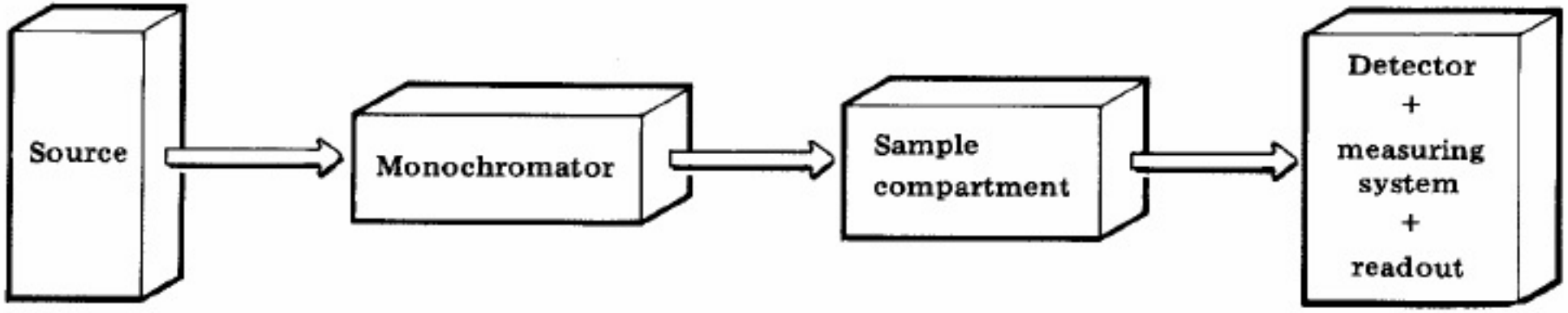
If a solution has this colour….

….it will absorb this colour….

UV-VIS instrumentation

The minimum requirements of an instrument to study absorption spectra (a spectrophotometer) are shown below:

1. a source of radiation of appropriate wavelengths.
2. a means of isolating light of a single wavelength and getting it to the sample compartment - monochromator and optical geometry.
3. a means of introducing the test sample into the light beam - sample handling.
4. a means of detecting and measuring the light intensity.

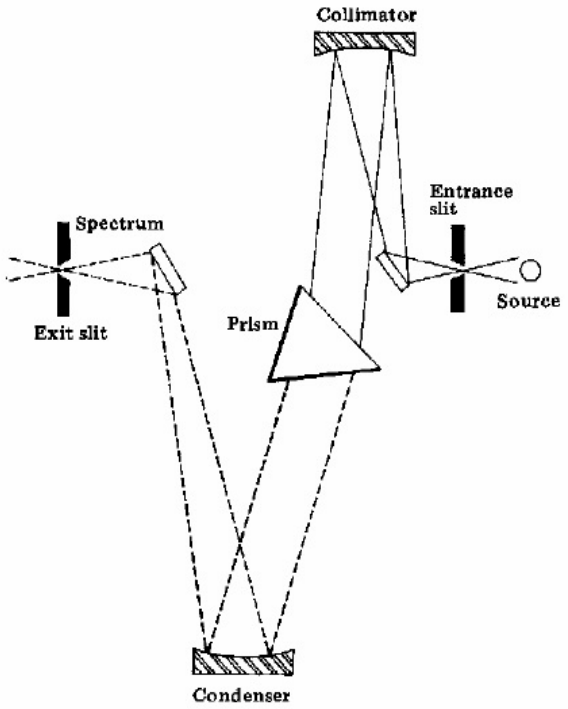


Light source & monochromator

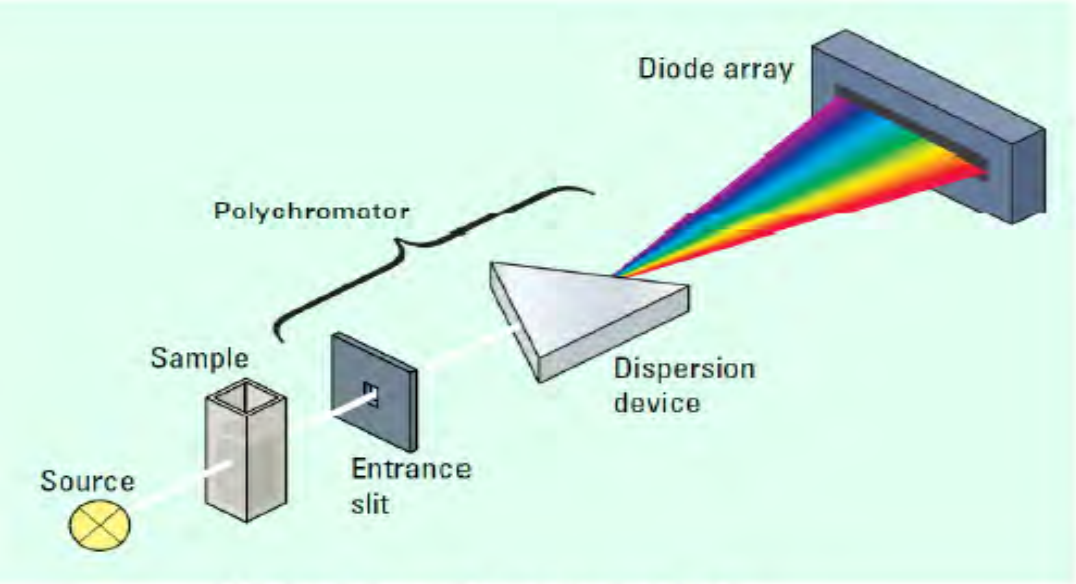
The requirements are that the source should be stable during the measurement period, i.e. that the intensity of emitted radiation should not fluctuate, and that there should be adequate intensity over as large a wavelength region as possible.

Ultraviolet light is generally derived from a deuterium arc that provides emission of high intensity and adequate continuity in the **190 - 380 nm** range.

Visible light is normally supplied by a tungsten lamp or, in modern systems, by a tungsten-halogen (also described as quartz-iodine) lamp which has higher relative output in the cross-over region (320 - 380 nm). The long wavelength limit is usually the cut-off of the glass or quartz envelope, normally well beyond the useful visible limit at **900 nm**

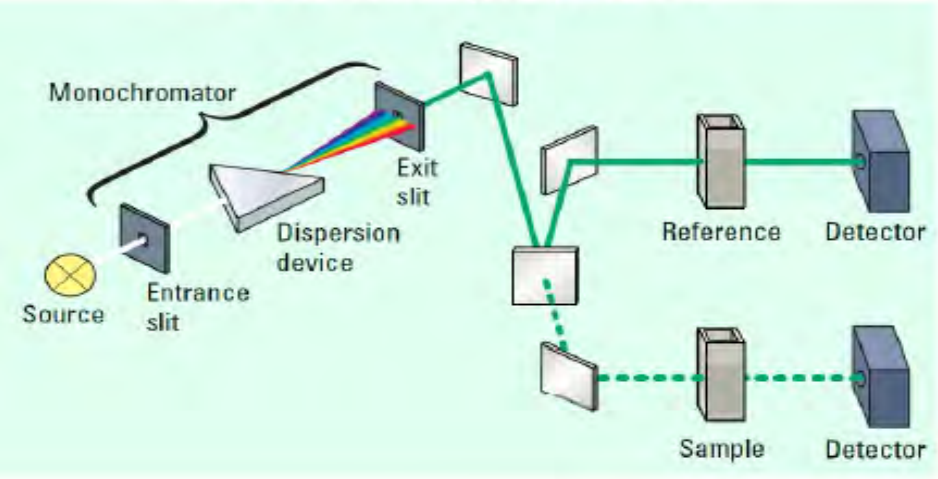


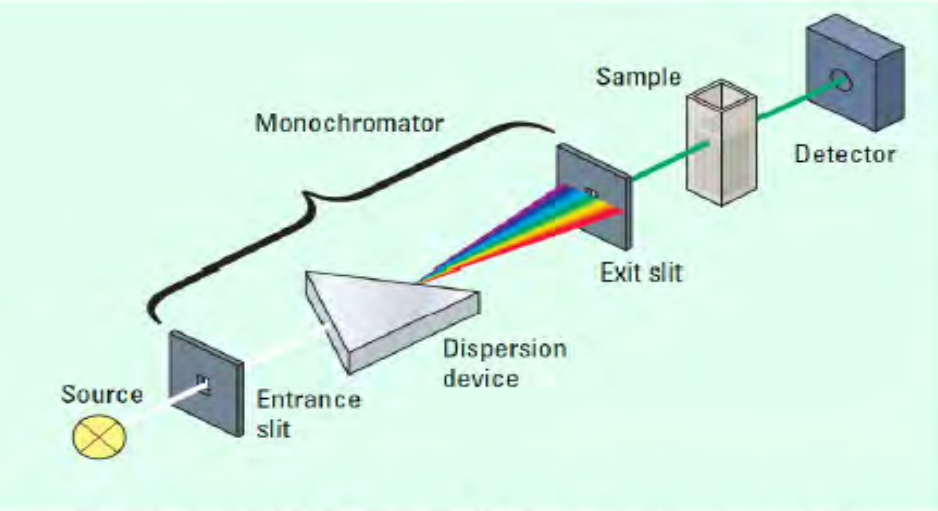
The function of a monochromator is to produce a beam of monochromatic (single wavelength) radiation that can be selected from a wide range of wavelengths. The essential components are: entrance slit, collimating device (to produce parallel light), a wavelength selection or dispersing system, a focusing lens or mirror and an exit slit.



Diode Array Spectrometer

Dual Beam Spectrometer





Conventional single beam spectrometer

ANALYTIK JENA SPECORD 250 PLUS

The devices optical properties/concepts and spectral resolutions:
0.5; 1; 2 and 4 nm, double-beam spectrophotometer with upstream monochromator

Technical data of the SPECORD 250 PLUS spectrometer:

Optical principle	Double-beam spectrophotometer with two tempered photo diodes (CDD; Cooled Double Detection)
Optics	Upstream monochromator and monochromator with holographic concave grid. Division into two beam paths through fixed 50/50 beam divider
Wavelength range	190 – 1100 nm
Photometric measuring range	-4 – 4 A (display range -8 – 8 A)
Photometric accuracy VIS at 546nm with neutral glass filter Hellma F4	≤ ±0.003 A
Photometric accuracy UV with potassium chromate according to Ph.Eur.	≤ ±0.01 A
Photometric reproducibility	≤0.0005 A

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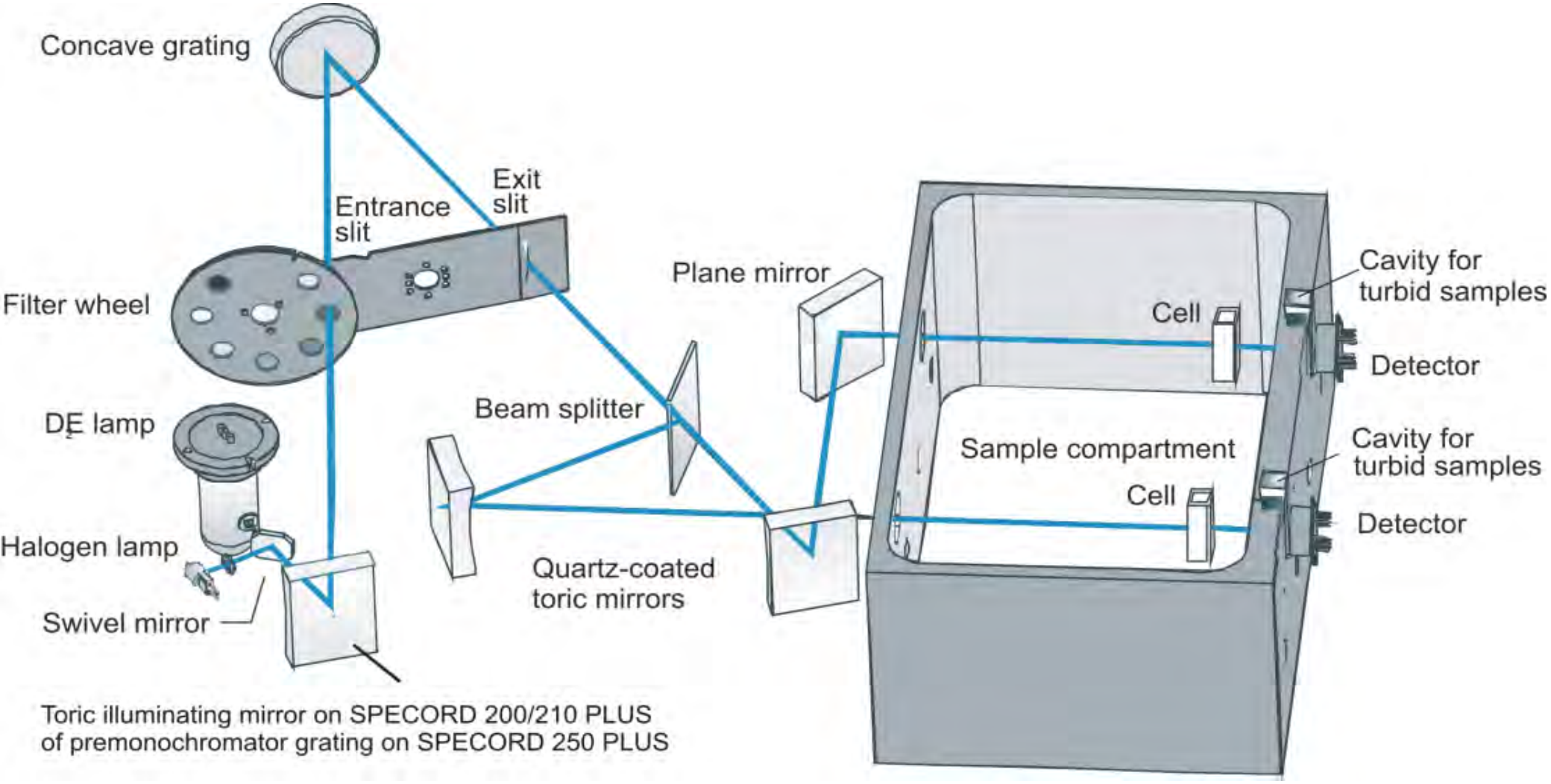
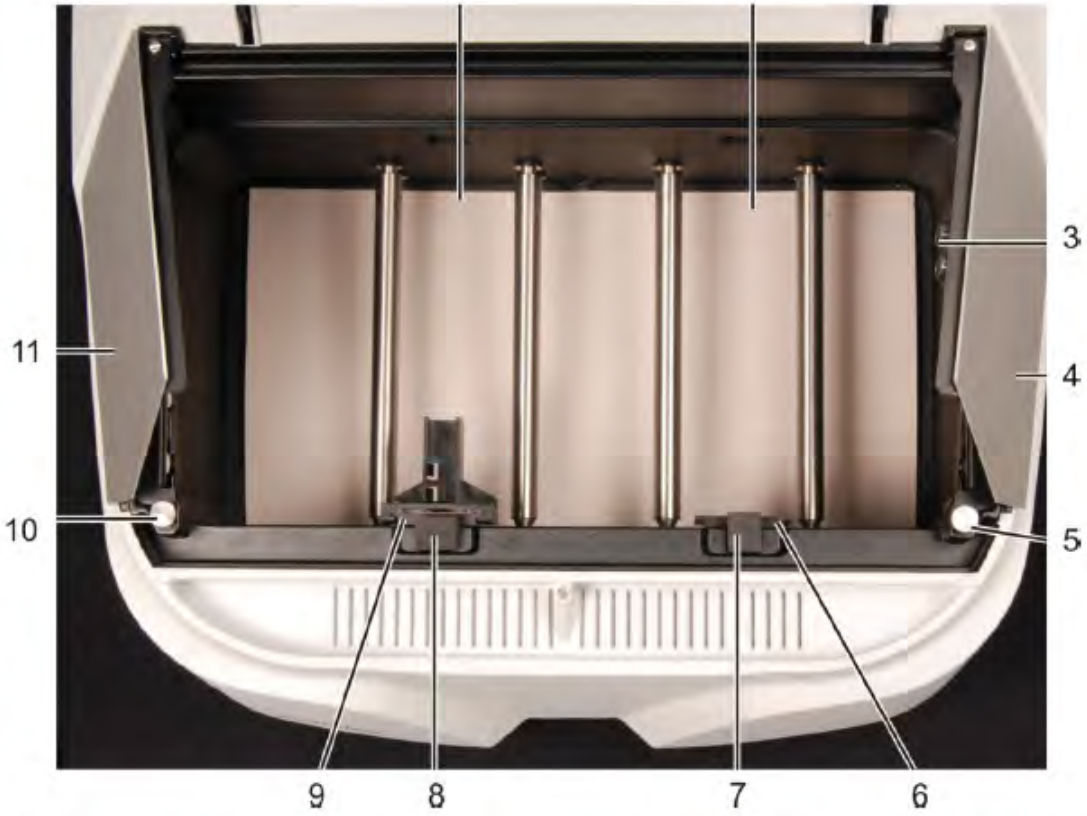
ANALYTIK JENA SPECORD 250 PLUS

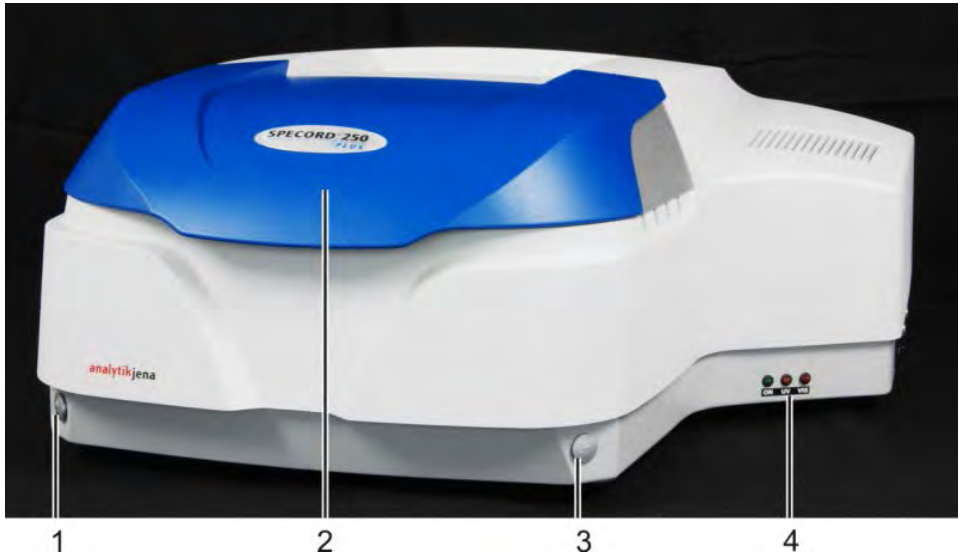


Toric illuminating mirror on SPECORD 200/210 PLUS
of premonochromator grating on SPECORD 250 PLUS

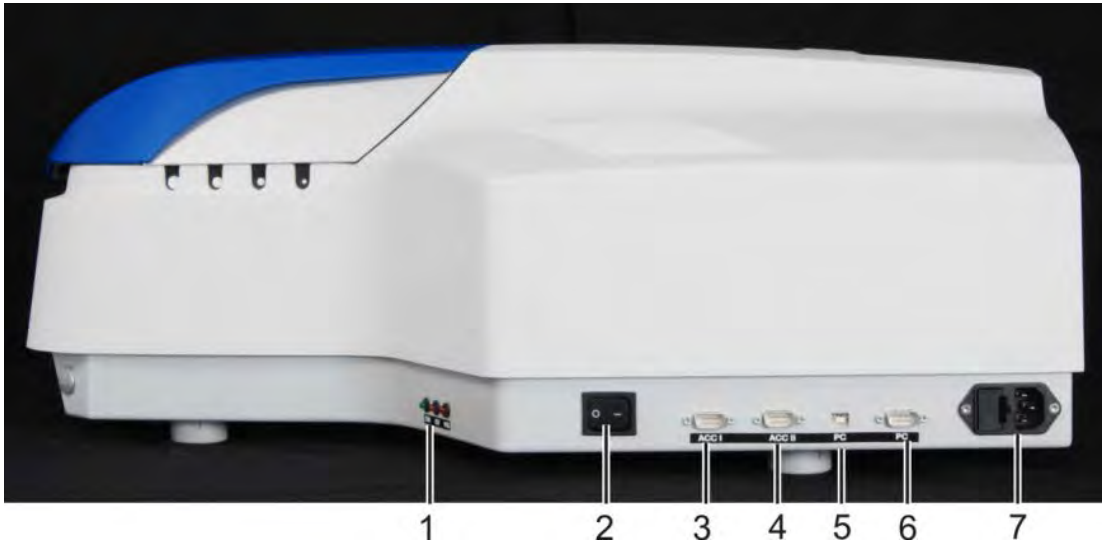


1	Measuring beam path	5, 10	Attachment screws for side components
2	Comparison beam path	6, 9	Plates to accept the cell holders
3	Connections for electrical accessories	7, 8	Cell ducts to accept dispersing samples
4, 11	Removable side components		

Sample chamber of the SPECORD 250 PLUS (from the top)



1, 3 Cut-outs for the waste hose of the sipper system and hoses of temperature-controlled cell holders and changers
2 Sample chamber cover
4 Status lamps for mains voltage, deuterium and halogen lamps



1 Status lamps
2 Mains switch
3 Connection for the control of a second Peltier tempered cell holder (ACC 1)
4 Connection for the control of a second Peltier tempered cell holder and the autosampler (ACC 2)
5 PC connection USB (PC)
6 Connection RS 232
7 Socket for mains connection and fuse holder

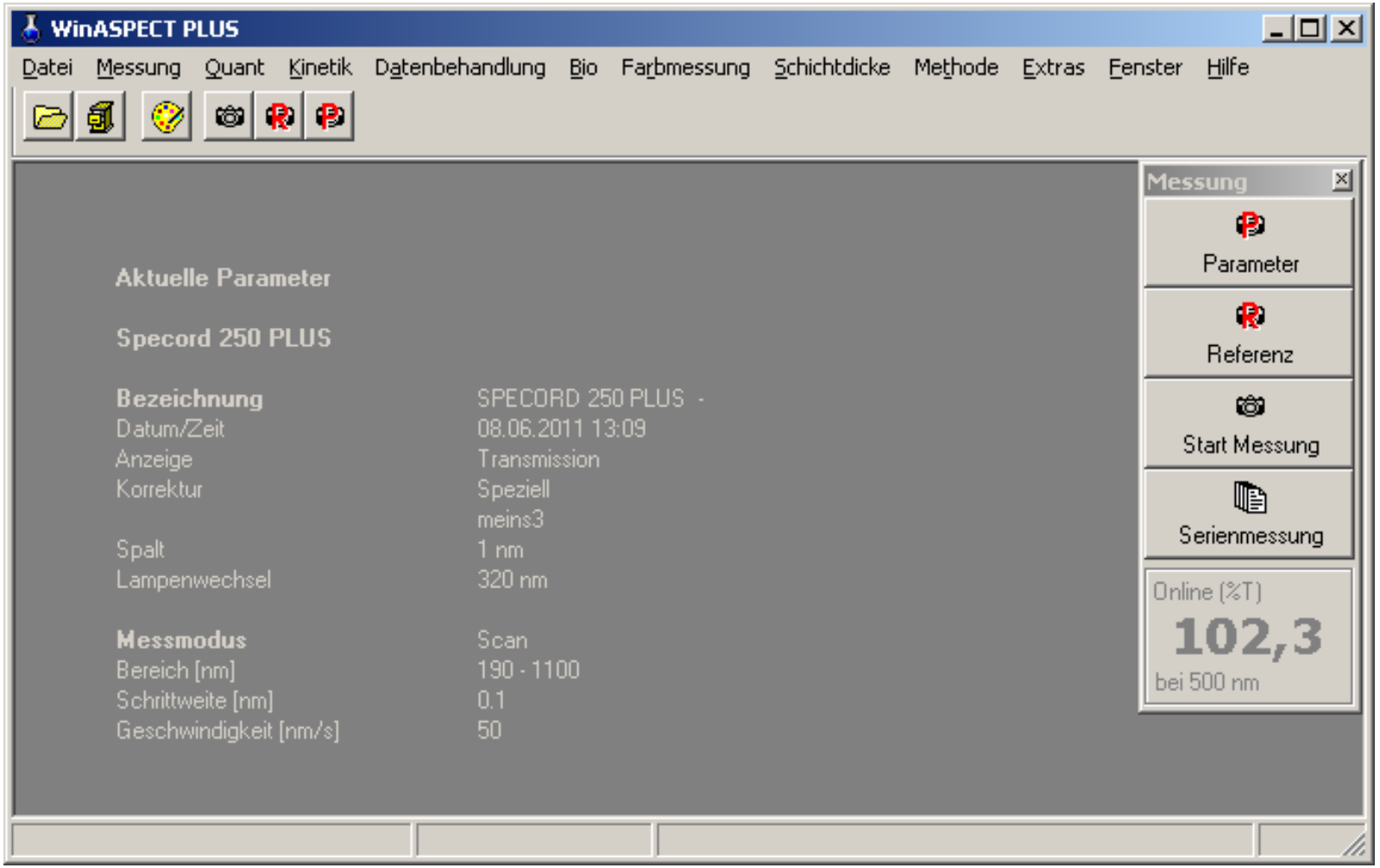
WinASPECT PLUS, Spectro-analytical Software

WinASPECT PLUS is a modular Windows software to control the UV-VIS spectral photometers of Analytik Jena AG and to display and analyze the measurements achieved using these devices.

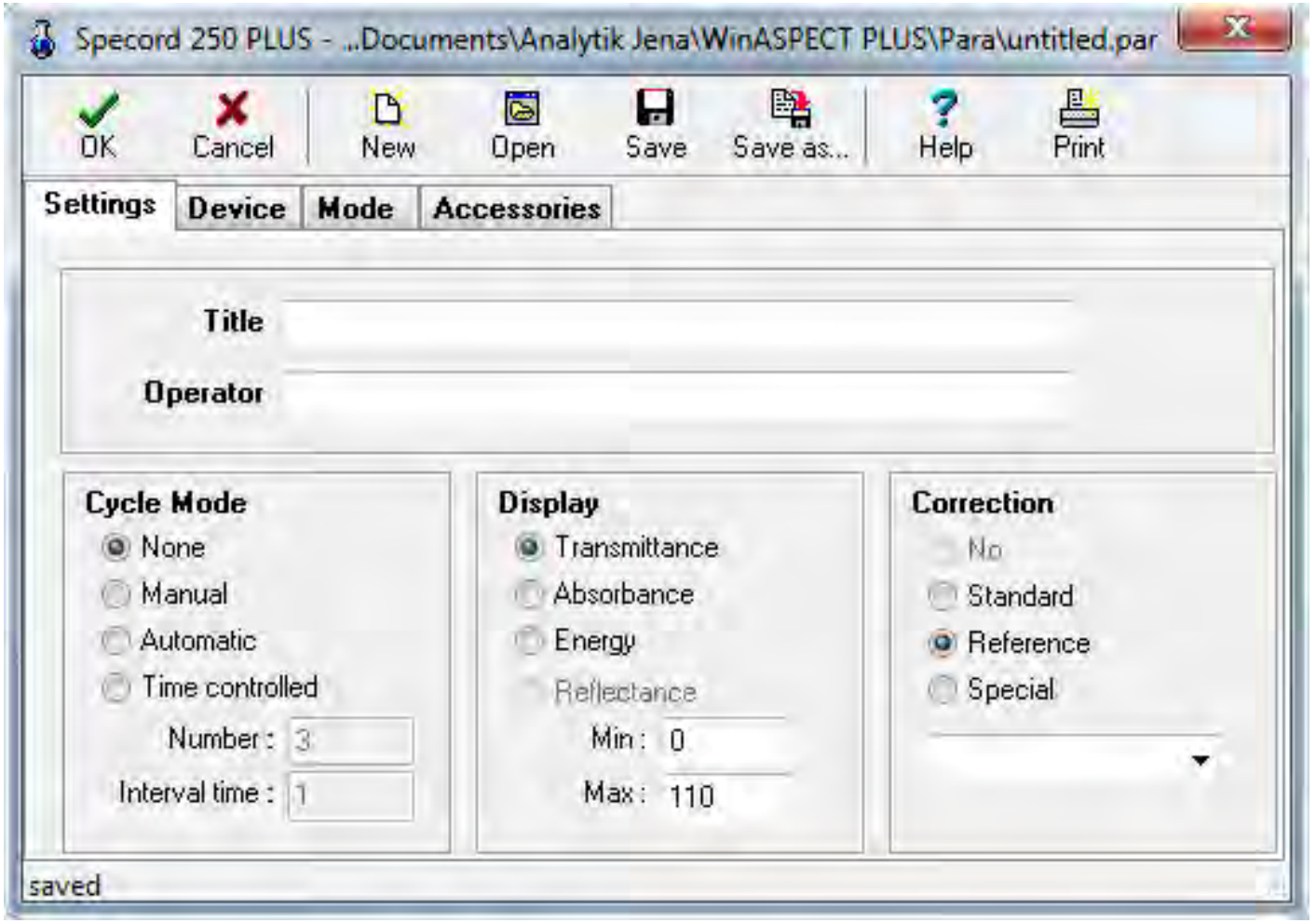
The following analysis modules have been implemented in WinASPECT PLUS:

- ❑ Quantitative analytics including a comprehensive set of calibration models and analysis
- ❑ Quantitative analytics for a routine with large sample throughput
- ❑ Kinetics
- ❑ Mathematical data handling
- ❑ Bio module for analyzing biochemical analyses
- ❑ Water analytics module for Spectroquant test kits by Merck
- ❑ Method Programming
- ❑ Color measurement
- ❑ Film thickness measurement
- ❑ Implemented methods for enzymatic examinations for medicine and food analysis
- ❑ Implemented methods for the analysis of beer

The WinASPECT PLUS workspace



The measurement parameter window



Calibration