Optimum Parameters for UV-Vis Spectroscopy

Agilent
Trusted Answers

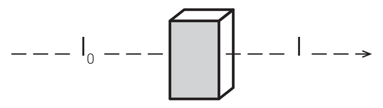
The Beer-Lambert Law

The concentration of a solute is directly proportional to the absorbance of the solution

Where Transmittance T is defined as
$T = I/I_0$ and $\%T = 100 \times I/I_0$

Where I_0 = Incident radiation

I = Transmitted radiation



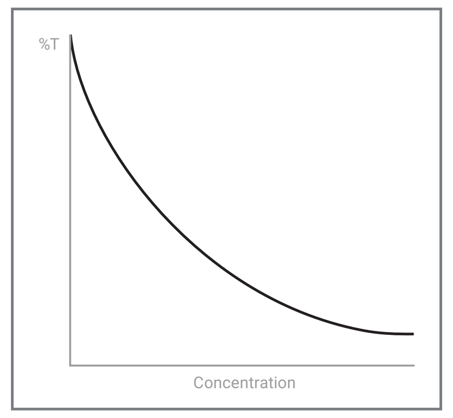
The Beer-Lambert Law defines Absorbance,
A, as $A = -\mathrm{Log}_{10}(I/I_0) = \mathrm{Log}_{10}(1/T) = ECD$
$= \mathrm{Log}\,(100/100 - \%\mathrm{Absorption})$

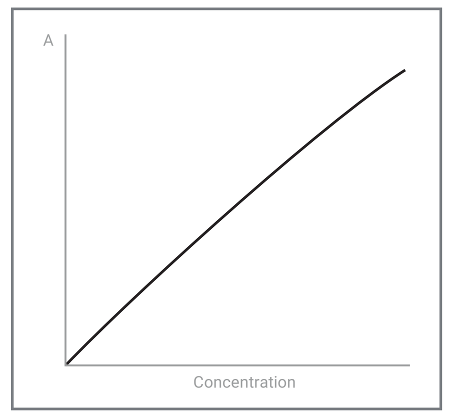
Where E = molar absorptivity, $M^{-1}cm^{-1}$
C = concentration of the solute, M
D = pathlength, cm

(Transmittance is often expressed as a percentage, so the relationship $A = \mathrm{Log}_{10}100/\%T$ is common.)

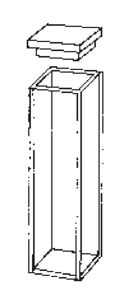
%Absorption = 100 – %T
Absorbance ≠ %Absorption

Absorbance is often more useful than Transmittance as there is a linear relationship between A and the concentration of the absorbing species. At higher concentrations there are deviations from linearity.

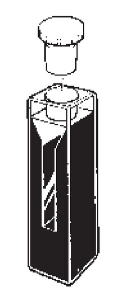




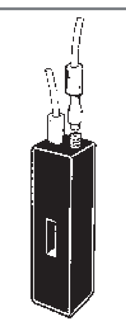
Optical Cells



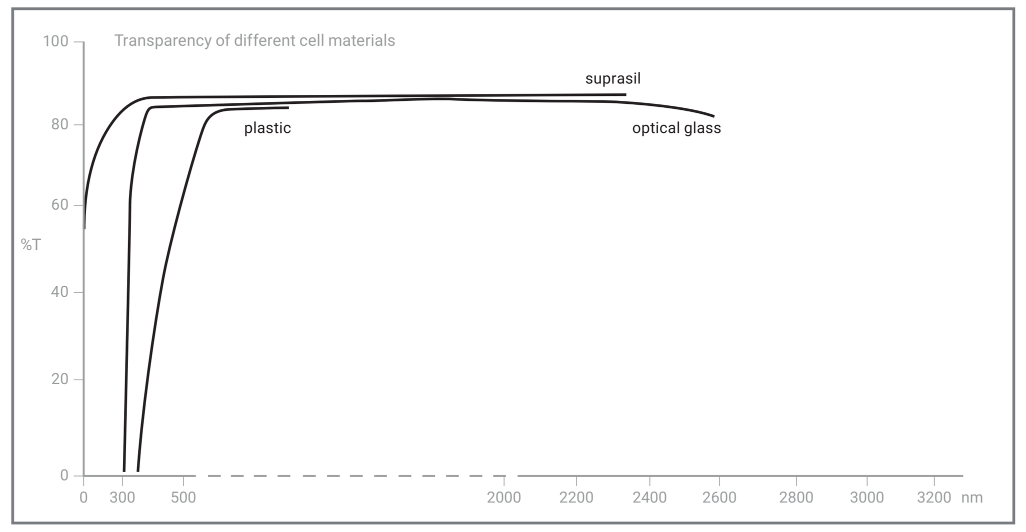
Standard
10 mm pathlength
For most materials



Microcell
10 mm pathlength,
2.5 mm path width
For limited or expensive samples

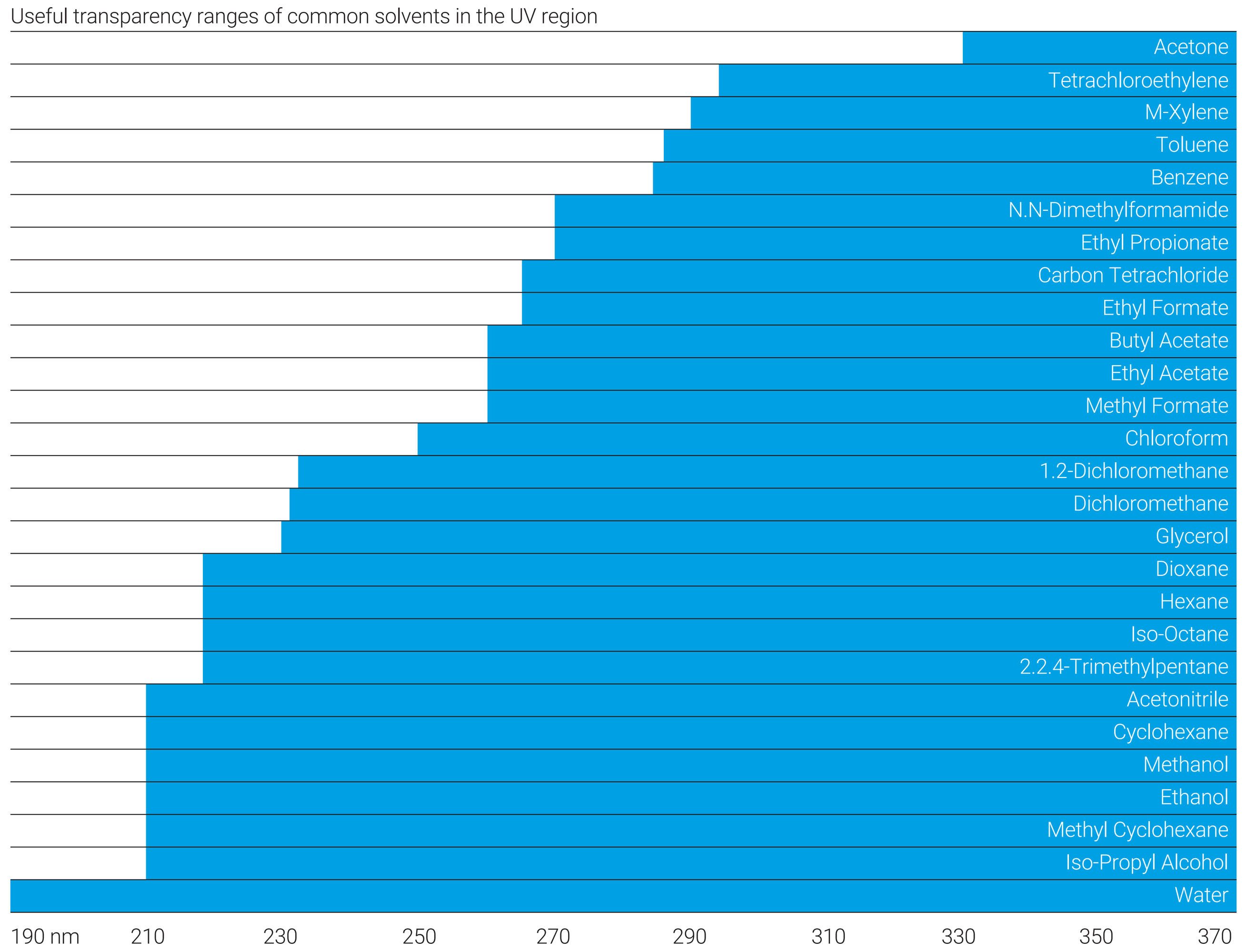


Flow cell
For measuring samples in a continuous flow



Solvent Transparency

Useful transparency ranges of common solvents in the UV region



Conversion Tables

%T	T	Abs	%A	LogA
100	10	0	0	–
50	0.5	0.3	50	-0.52
10	0.1	1	90	0
1	0.01	2	99	0.3
0.1	0.001	3	99.9	0.48
0.01	0.0001	4	99.99	0.60
0.001	0.00001	5	99.999	0.70
0.0001	0.000001	6	99.9999	0.78
0.00001	0.0000001	7	99.99999	0.85

λ	cm^{-1}
3300	3030
3000	3300
2500	4000
2000	5000
1500	6666
1000	10000
800	12500
600	16667
400	25000
200	50000
175	57143

Cary 3500 UV-Vis Spectrophotometer

Multiply Your Experimental Possibilities

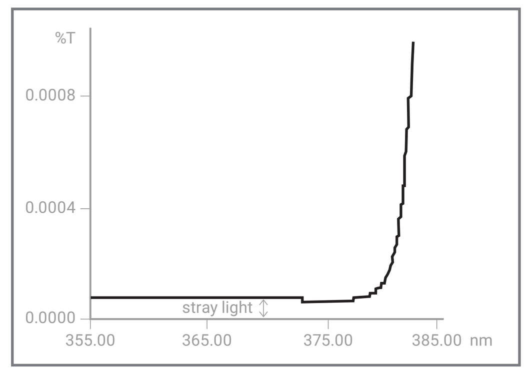
Instrument Features – Why They Matter

Stray Light

Wavelengths of light other than the desired wavelengths that reach the detector.

How it is measured: The solutions used to test stray light levels are non-transmitting at the indicated wavelengths (they do transmit at other wavelengths), so the observed transmittance is due only to stray light.

The effects: The stray light level directly influences the maximum absorbance the instrument is able to measure. Stray light also causes deviations from the Beer-Lambert Law.

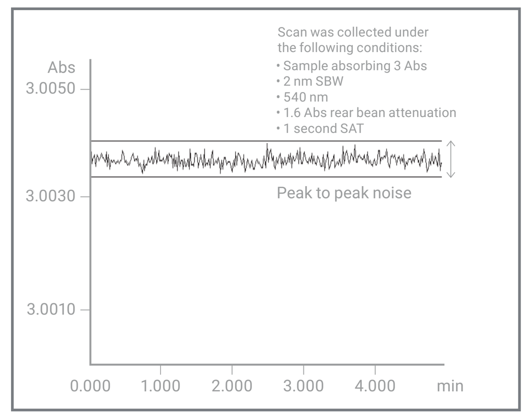


Noise/Speed

The 'noise' level is an indication of how stable a reading is. It determines the precision of the measurement and the detection limit of the instrument. Lower noise levels permit faster scan rates at equivalent precision and detection limits.

How it is measured: Noise is measured at a particular wavelength and Absorbance level. It is either specified as the peak-to-peak value (maximum deviation on either side of the Abs value) or the RMS (root mean square) which is approximately one fifth of the peak-to-peak value.

The effects: Poor signal-to-noise performance makes it very difficult to tell what the real Abs value is as it fluctuates. It introduces errors into both quantitative and qualitative spectroscopy.

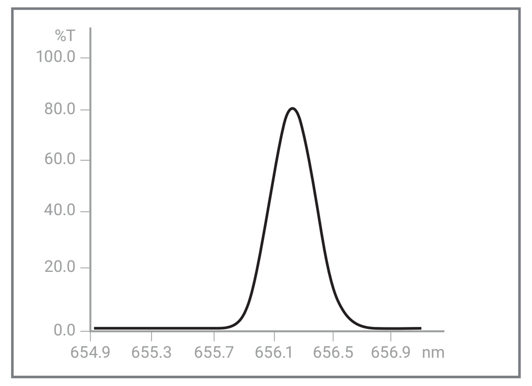


Wavelength Accuracy

How close the indicated wavelength is to the actual wavelength.

How it is measured: By scanning the Xenon (Xe) and Deuterium (D_2) emission lines and observing the wavelengths at which these lines are measured.

The effects: Poor signal-to-noise performance makes it very difficult to tell what the real Abs value is as it fluctuates. It introduces errors into both quantitative and qualitative spectroscopy.

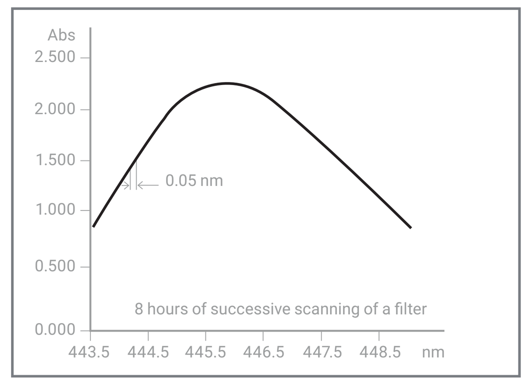


Wavelength Repeatability

The ability of the instrument to correctly return to the set wavelength repeatedly.

How it is measured: By repeatedly scanning emission lines(s). If the wavelength repeatability is good, exactly the same trace should be obtained each time.

The effects: Poor wavelength repeatability will result in errors in quantitative analysis due to wavelength shifts.

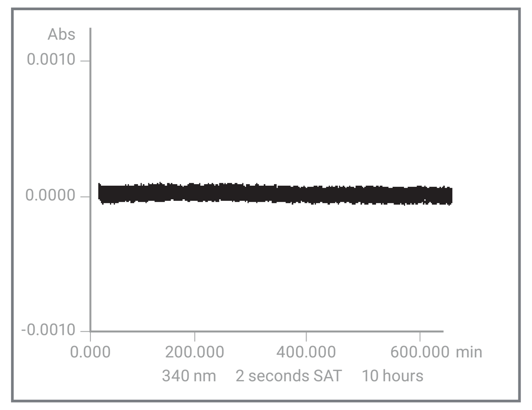


Long-term Stability

The drift of the reading per unit time.

How it is measured: By recording the Abs of a sample at one wavelength for several hours. The specification indicates the maximum deviation from the correct Abs value per unit time.

The effects: Poor stability introduces errors in time-based measurements. The instrument cannot distinguish between slope due to reaction or slope introduced by the instrument. Baseline correction can be invalid.

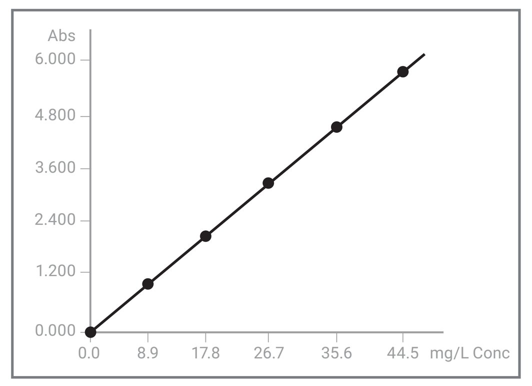


Photometric Linearity

How accurately the instrument measures absorbance with increasing concentration.

How it is measured: By measuring the absorbance of successive $K_2Cr_2O_7$ solutions of increasing concentrations, or by measuring the additive absorption of a series of filters.

The effects: Poor photometric linearity will produce incorrect results.



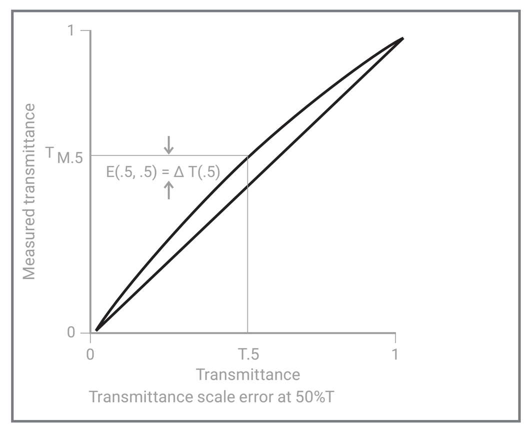
Photometric Accuracy

How accurately the instrument measures.

How it is measured: Photometric accuracy has traditionally been measured by the following methods:

Chemical standards, such as $K_2Cr_2O_7$ (accuracy ±0.005 Abs) calibrated neutral density filters (accuracy is within 0.5 to 1% of their stated values or 0.002 to 0.004 Abs). The Double Aperture method is another accurate method. It is used by major national standards laboratories to measure the absolute accuracy of their reference spectrophotometers. This method has no limitations on spectral band width, wavelength or temperature and can yield precisions about 2 orders better than the previously mentioned uncertainties.

The effects: Poor photometric accuracy will produce incorrect results.



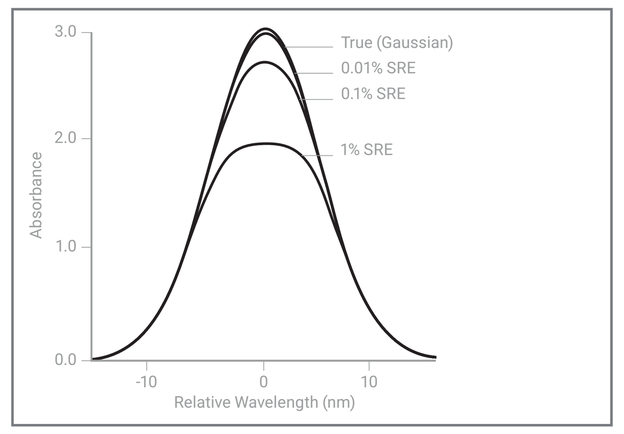
Optimum SBW For Typical Compounds

Representative Compound	Peak nm	Band Width nm	Optimum (SBW) nm
Amino Acids			
tryptophan	279	45	4.5
tyrosine	275, 195	40, 10	4.0, 1.0
phenylalanine	258	2.2	0.2
Nucleotides			
adenosine	260	28	2.8
thymine	265	30	3
Proteins			
cytochrome c, oxidized	410	25	2.5
rhodopsin	500, 278	~90, 25	9, 2.5
ribonuclease	278	~20	2
Pigments and Dyes			
ß-carotene	480	35	3.5
chlorophyll a	660	20	2
Coenzymes			
Nicotinamide adenine dinucleotide	260	35	3.5
NADH	340, 260	50, 25	5, 2.5
Simple Organics			
benzene, vapor	253	<<0.1	<<0.01
benzene, solution	253	2	0.2
anthracene	375	3	0.3

Instrument Parameters

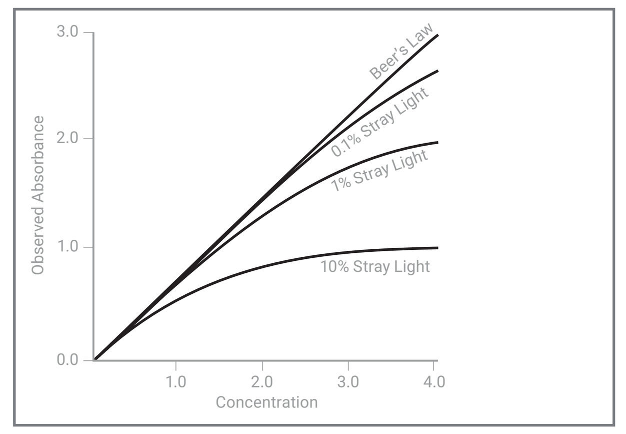
Stray Light

Stray light or stray radiant energy (SRE) is defined as the percentage of radiation reaching the detector whose wavelengths are outside the selected spectral band.



The effects of SRE are:
- decreased absorbance readings
- changes in band shape.

The level of SRE determines the maximum Abs measurable by the instrument.



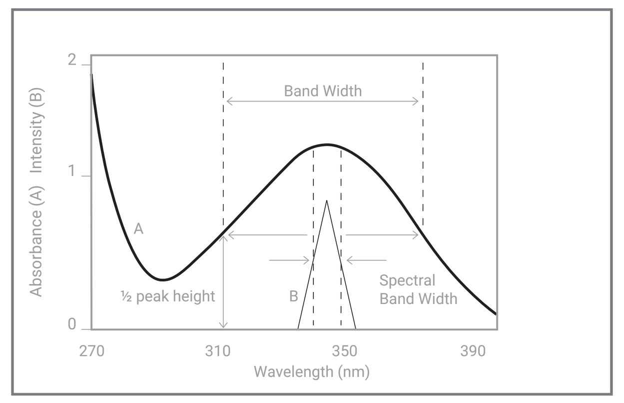
This causes deviation from the Beer-Lambert Law.

Resolution

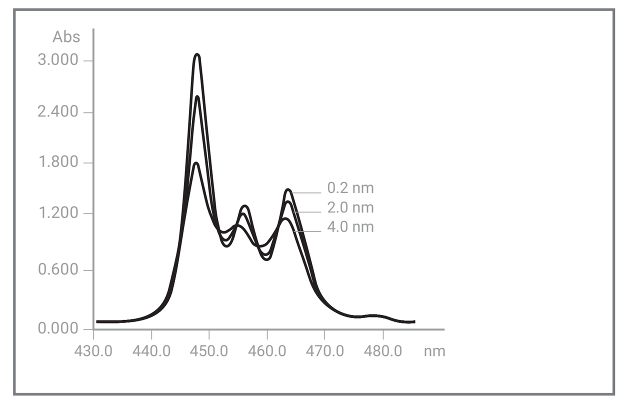
Spectral Band Width should be set to one-tenth of the band width.

Resolution the spectrophotometer's ability to distinguish between two absorbance bands which are close together.

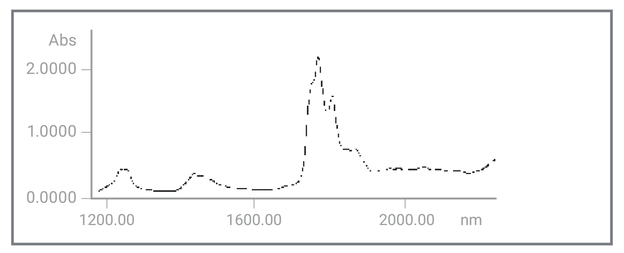
Data Interval a minimum ratio of SBW: Data Interval of 3:1 should be set to ensure that no spectral detail is lost.



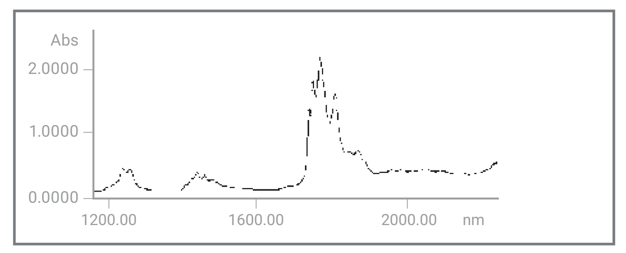
The band width is the width of the peak at half peak height.



As the slit widens the signal-to-noise increases but the resolution decreases.



These two scans demonstrate the effect of varying the SBW.
The above graph shows a scan of hexane with a SBW of 15 nm.



This graph shows the same sample scanned with a SBW of 3 nm – with considerably better resolution.

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