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**A NATIONAL MEASUREMENT
GOOD PRACTICE GUIDE**

No. 97

Regular Transmission
Measurements



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Measurement Good Practice Guide No. 97

Regular Transmittance Measurements

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ABSTRACT

This document is a guide to the assessment of spectrophotometers that are used to make regular transmittance measurements of optical radiation at ultra-violet (UV), visible and near infrared (NIR) wavelengths from 200 nm to 3000 nm. Good practice is covered along with guidance on the assessment of uncertainties in the measurements made.

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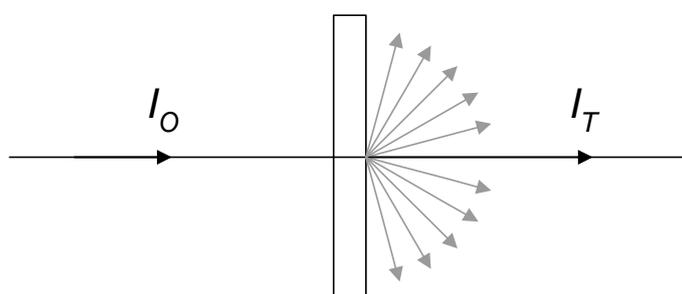
Introduction

1

This guide is concerned with measurements of regular transmittance in the near ultraviolet, visible and near infrared spectral regions.

Regular transmittance is the ratio of the intensity of the regularly transmitted part of the incident radiation to the incident radiation. Regularly transmitted radiation is that which has been transmitted through a sample without being diffused (scattered), and is illustrated in Figure 1. Generally the type of samples measured for regular transmittance may absorb some or all of the incident radiation, but any transmitted, is a sharp image of the incident beam, i.e. the sample does not diffusely scatter the light. The sample may be a solid, liquid or gas.

Figure 1: Regular transmittance $T_x = I_T/I_0$: I_0 incident light, I_T transmitted light



Regular transmittance measurements are the basis of a number of other quantities used in spectrophotometry, such as optical density, absorbance, molar absorptivity, and dosimetry.

Industries that use transmittance measurements include pharmaceuticals and analytical chemistry, plastics, glass, optical materials and coatings, optical device manufacturers etc.

The accuracy of any measurement will depend on the design of the measuring instrument and the quality of the sample. In order to ensure that measurements are meaningful, best practice needs to be followed. This guide attempts to show what is needed in ensuring best practice.

Quality Assurance

2

Regulation and the need to have compliance with quality system procedures are becoming almost universal. Regular transmittance measurements are usually made using a spectrophotometer. A spectrophotometer is able to make these measurements at specified wavelengths within its spectral range. There is the need to show that the spectrophotometer is functioning correctly and is fit for the task (*design qualification*)^[1, 2], and is compliant with the manufacturer's performance criteria (*installation qualification*). The spectrophotometer needs to comply with established standards and user practices, and test procedures set up (*operational qualification*) and have documented evidence for continued operability and data integrity (*performance qualification*). In addition, measurements may also be required to demonstrate traceability to the SI or national standards.

This means knowing what sort of samples are to be measured, what accuracy and measurement resolution are needed, whether the chosen spectrophotometer is capable of the measurements demanded of it, what the limitations of the spectrophotometer are, and the uncertainty needed for the final measurement result.

Manufacturers usually provide a specification of the spectrophotometer's capabilities which determines whether the instrument can in theory be used to make the necessary measurements. If this is so the user then needs to verify that the instrument is operating within the manufacturer's specification.

Additionally issues such as reproducibility of measurements between similar or different instruments in a laboratory and reproducibility of measurements between laboratories, may also need to be considered.

For organisations operating with a quality system further guidance can be found in ISO 17025, General requirements for the competence of testing and calibration laboratories^[3]. For test and calibration facilities that undertake health and environmental safety studies and other testing that will be submitted to regulatory authorities for the purpose of risk assessment, compliance with Good Laboratory Practice Regulations will also be needed^[4].

Traceability

3

Measurements should be traceable to the *System International* (SI). A country may realize its own scale for spectral transmittance, or may obtain traceability to the SI by using the scale of another country. Regardless of this, for the end user to establish traceability, reference standards which are traceable to a national metrological institute, and thereby to the SI, must be used to validate the measurement technique employed^[5, 6].

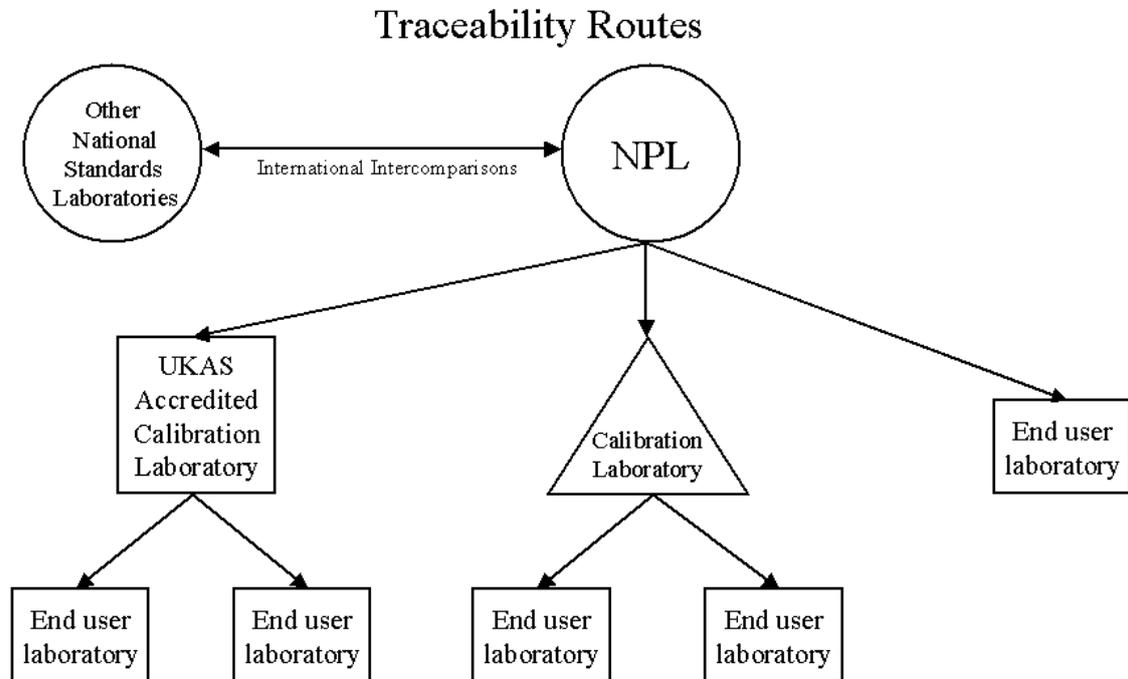
National metrological institutes, which offer transmittance traceability, will have a reference instrument in which systematic uncertainties have all been carefully evaluated. Two quantities in spectrophotometry need physical standards: the photometric (or intensity) scale and the wavelength scale. In addition the stray radiation in the spectrophotometer needs to be known.

Reference standards should be recalibrated at regular intervals at a national standards laboratory, or at an appropriately accredited (e.g. UKAS accredited) or in-house calibration laboratory that can demonstrate traceability to national standards. The laboratory should be capable of making calibrations at an uncertainty level appropriate to the measurements being made. Any certificate for a standard should give an uncertainty at a stated confidence level, most usually 95%. If the measurements on a reference standard agree with the reference standard values within the defined combined uncertainty of the standard and the spectrophotometer then the measurement technique is satisfactory for that type of measurement.

Reference standards from national laboratories are often solid transmitting materials as these are generally more stable, robust and easier to handle than liquids. Analytical chemists make the majority of their measurements on liquids. This raises the question of whether solid standards are appropriate for chemistry. Where medium accuracy (accuracy/transmittance = 0.005 - 0.02) or low accuracy (> 0.02) is needed, liquid standards may well be the appropriate choice. Solid standards are generally preferable to liquid standards for high accuracy (< 0.005) work, as they are more stable and the calibration values are not dependant on the expertise and skill of the person preparing the solution in any given laboratory. Liquid standards require care to be taken with pipettes used to transfer solutions in and out of the cuvette. The cuvette may also affect the measurement. For example the alkalinity of soft glass may affect PH sensitive solutions. However some liquid reference standards are now available premixed in sealed cuvettes.

When using solid transmitting standards it is good practice to use an identical empty holder for reference measurements. This is analogous to using a reference solution when using liquid standards.

When using reference standards, consideration has to be given to their care and storage. Standards may be susceptible to temperature, exposure to light, physical damage, chemical corrosion and chemical decomposition. For example, sodium iodide and sodium nitrate show temperature induced chemical decomposition.

Figure 2: Traceability routes from NPL

Regular transmittance measurements

4

IN THIS CHAPTER

- Cascade measurements for low transmittance materials

Before discussing the use of spectrophotometers, it is worth considering regular transmittance measurements in the most general sense.

Figure 1 shows that two measurements are required, the intensity I_O of the incident beam, and the intensity I_T of the transmitted beam. Transmittance is then given by I_T/I_O . The simplicity of this scheme nonetheless requires that care be taken in carrying out this measurement.

Some factors that may lead to invalid measurements are listed below. Some the user may be able to control and others not, depending on the spectrophotometer design. The interested reader is directed to an extensive literature which discusses these more fully – see Sections 9 and 10.

1. The spectrophotometer user should ensure that at the detector all of the beam cross-section falls within the detector active area for both the I_O and the I_T measurements.
2. If the detector is not spatially uniform, then insertion of the sample should not alter the beam focus or deviate (e.g. by refraction) the I_T beam position on the detector from the I_O beam position. This may appear to be solved by setting the sample at normal incidence, but see 3. below.
3. If the beam is normally or near-normally incident on a parallel-faced sample, inter-reflection effects can occur between sample and detector and between sample and pre-sample components, including the source (see section 5.7).
4. If the sample is placed at a slit image that is in turn focused on the detector surface, then any optical imperfections as mentioned in 2. or 3. above will have minimal effect, as compared with when the sample is placed away from such a focus or in a collimated section of beam. The effect will be further minimised if the relay focusing optics between sample and detector are under-filled by the beam.
5. The detector (or more generally the output signal after any electronic processing) should have a response that is an exact linear function of the radiant power incident on it. Any deviation from this ideal should be quantified and corrected for, or alternatively eliminated by adjustment or modification to the electronic circuits involved.
6. The response from the detector system should be recorded when the radiation from the sample beam is blocked with an opaque matt black obstruction, giving a zero-offset reading, which should be algebraically subtracted from both I_O and I_T measurements.
7. The introduction of the sample must not shield any stray radiation from reaching the detector.
8. Unless the user is certain that the transmittance of the sample does not vary with wavelength significantly over the spectral range of measurement, the errors of the wavelength scale need to be evaluated and corrections applied.

4.1 Cascade measurements for low transmittance materials

For measurements made on samples with transmittances of less than 10% there may be issues of low signal-to-noise level with consequent increase in measurement uncertainty. The uncertainty can be reduced by making measurements against a previously measured reference sample, which has a transmittance of no more than ten times that of the sample being measured. This technique allows the full scale of the spectrophotometer to be used. This is achieved by putting the reference into the spectrophotometer and running a baseline to set the 100% level. The reference is removed and the sample measured. The results obtained for the sample are relative to the reference. The absolute sample values are then obtained by multiplying the sample by the reference values expressed as a decimal.

Multiple cascades can be made. For example to measure a filter of 0.1 % transmittance, a 10 % filter is measured against air, then a 1 % filter is measured against the 10 % filter, and finally the 0.1 % filter is measured against the 1 % filter.

Assessment of the spectrophotometer

5

IN THIS CHAPTER

- Specification
- Geometric considerations
- Instrument start up
- Linearity of the measurement system
 - » Neutral density filters
 - » Liquids
 - » Beers law
- Repeatability
- Reproducibility
- Sample induced errors
 - » Interreflections
 - » Fluorescence
- Polarisation
- Wavelength Scale
- Stray light
- Bandwidth
- Cleaning of standards
- Short- and long-term aging of spectrophotometer

The ideal instrument has constant (wavelength independent) source output, monochromator efficiency and detector efficiency, combined with no polarization effects, linear signal response, perfect wavelength accuracy, perfect wavelength resolution, short term source stability and constant output over the source's lifetime.

Spectrophotometers come in a variety of configurations. Instruments are usually of the dispersion type, based on the use of diffraction gratings. Fourier transform (FT) instruments are starting to be used at visible and near infrared wavelengths. Their measurement characteristics and problems^[7] are so distinct from those of grating instruments that they are not considered in this Guide. Grating instruments can be either single or double beam, have one or more light sources and detectors, have diode array detectors or separate detectors, scan over an extended range of wavelengths, or have a fixed wavelength range. Many instruments have their own internal calibration routines and/or calibration artefacts. However, it is advisable to obtain other calibration artefacts to independently check the instrument performance, or at least ensure that the supplied artefacts have been traceably calibrated.

Some modern computer controlled spectrophotometers emphasise the display and manipulation of data without due regard for the quality of the underlying data. This can hide the inadequacies of measurement and a lack of understanding of the sample.

The variety of spectrophotometers used means that some or all of the following need to be considered when assessing a spectrophotometer.

5.1 Specification

Ideally the process of assessing a spectrophotometer starts before an instrument is purchased^[2]. The user needs to have a clear idea of what measurements, uncertainties, wavelength ranges, etc. are required. This will ensure that the manufacturer's specification for the spectrophotometer can be compared to the user's requirements, and appropriate spectrophotometers short-listed for consideration of other criteria.

The spectrophotometer manufacturer will provide a performance specification for the spectrophotometer. This may include wavelength range, wavelength intervals, wavelength accuracy, type of illumination sources, source stability, warm up time, source lifetime, checks on source energy, transmittance range, absorbance range, optical density range, photometric linearity, measurement repeatability, inter-instrument agreement, operating temperature, cost etc. There may also be data on accessories that can be used with the spectrophotometer such as cell changers, filter holders etc. Most new spectrophotometers also have start up self-test routines to ensure the instrument cannot be used unless it is fully functioning.

Over the life of the instrument there is a need to ensure the spectrophotometer continues to meet the specification. The recording of environmental conditions of cell, sample compartment, laboratory etc., will help to identify long term changes that are occurring to the spectrophotometer and to identify causes of any anomalous effects that may occur.

5.2 Geometric considerations

The layout of the optical components in the spectrophotometer has a bearing on the measurement resolution and accuracy^[1]. The biggest geometrical difference is between a single beam and a double beam instrument. Single beam instruments assume the illuminating source and detector responsivity are stable during the measurement cycle. Double-beam instruments split the illumination into two paths with the sample under consideration in one path and the other path used for comparison. The instrument alternates very rapidly between the two paths and compares one beam against the other. This cancels out fluctuation or drift with time in the illumination intensity or detector response.

Consideration also has to be given to where the light dispersing element is placed in the system. This is usually a diffraction grating but could be a prism or a series of filters. Light dispersion could happen before the sample, resulting in the sample being illuminated with a small band of wavelengths, or after the sample, in which case the sample is illuminated with the full wavelength range. This has implications for the type of detectors used in the system, and for effects that may occur if the sample is fluorescent^[8]. Instruments with light dispersion before the sample tend to be scanning instruments that have a single detector output. The detector is sensitive to a range of wavelengths. Instruments with dispersion after the sample usually have multiple detector outputs, e.g. from a diode array, mapped to the wavelength range of the instrument. Another point to consider is that heating and thermochromism effects may give measurement problems when the sample is directly irradiated with the full wavelength range of the source.

With double-beam instruments it is possible to directly compare a sample solution in a cuvette in one beam with a reference solution in another cuvette in the other beam and hence measure the differences between the two. This arrangement could give path length inaccuracies if the cuvettes are not a closely matched pair with the same properties of path length and beam deflection. Path length inaccuracies can also occur in a single beam instrument using separate cuvettes measured sequentially. If high accuracy is required the user may be better off using the same cuvette for both the sample and reference measurements, with thorough cleaning between using different solutions.

In diode-array detector systems, filter and cuvette faces must be parallel to each other to ensure light rays are not shifted along the array; otherwise the result will be an apparent wavelength error in the measurement.

Thought may also have to be given to the blocking of unwanted components of radiation throughput, such as second-order effects from gratings, interreflections within the optical system, fluorescent emissions, etc.

5.3 Instrument start up

Perform the start up routine as indicated by the instrument manufacturer. Nowadays this is automatically done by most spectrophotometers when switched on.

The time period elapsing for stable results should be determined. Stable results occur when the change in the instrument reading from one reading to the next is within acceptable limits.

The instrument manufacturer may suggest a warm-up time. Generally, let the instrument warm up for a time period that has been estimated to give stable displays/results, but never less than the time suggested by the spectrophotometer manufacturer or thirty minutes.

5.4 Linearity of the measurement system

Photometric/intensity accuracy of a spectrophotometer requires either a linear instrument response or accurate knowledge of the deviations from linearity. A linear instrument response means that, over a specified range, the output signal of the photodetector system is exactly proportional to the input signal, where the usual input and output signals are the radiant flux and the indicated output respectively. A spectrophotometer measures, at each wavelength, a ratio of radiant fluxes so any deviation from linearity will cause a direct error in the measured transmittance (or absorbance etc.).

Any non-linearity in the output of the detector system, i.e. detector and associated electronics, will change the measurement results. A detector such as a photomultiplier can be non-linear due to the way it detects and amplifies the signal. Saturation at high signal intensities can occur in photomultipliers and lead sulphide detectors. Lead sulphide detectors may not be linear at or near zero intensity. In the visible region silicon photodiodes have very good linearity. It is hoped that the spectrophotometer manufacturer has carefully selected the actual detectors and associated electronics to minimise non-linearities.

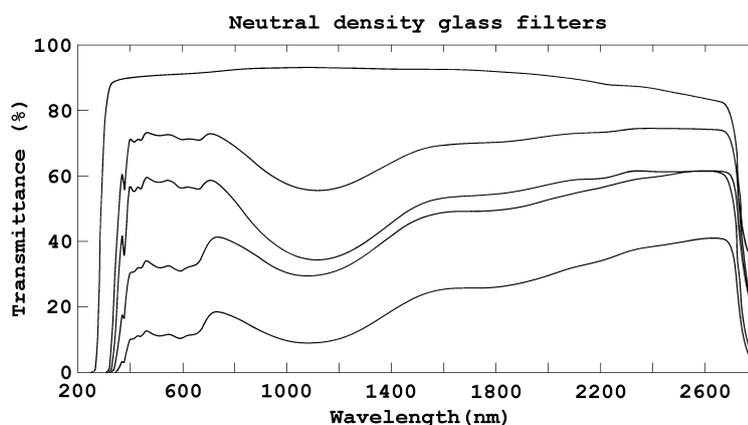
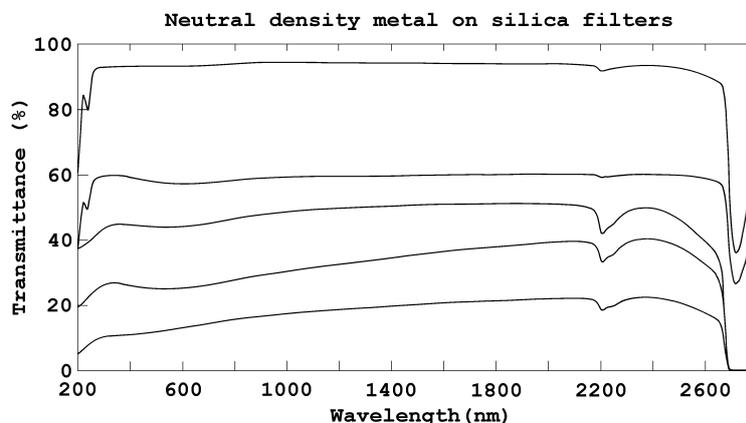
In regular transmittance mode consider the extremes of the detector system scale; the 100% level is measured with no sample in the beam, i.e. through air, and assigned the value of 100.0%. The 0% level is set with a blackened opaque sample that is the same size and shape as the test specimen and blocks the instrument sample beam. The measured zero level must be subtracted from all subsequent readings.

The best way of checking the linearity is to use a double aperture technique where a light beam is split into two parts that are measured separately and together^[9-11]. Any difference between the sum of the separate measurements and the combined measurement gives an indication of the non linearity for a particular intensity. This can be repeated at several intensities covering the measurement range.

Inserting a double aperture assembly inside a commercial spectrophotometer is not always practical, so the user usually has to rely on other techniques such as using calibrated transfer standards to assess the linearity of the measurement system. The transfer standards are usually a range of neutral density filters or standard solutions. The reference values for these should be traceable by an unbroken route to a national standards laboratory.

5.4.1 Neutral density filters

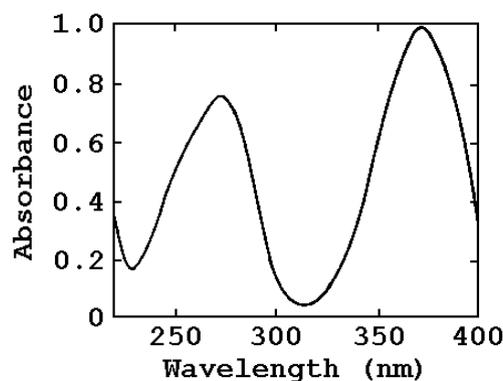
Filters to assess linearity should be neutral with wavelength to separate ordinate scale errors from wavelength errors^[12-14]. The filters should as a minimum cover the range of absorbances or transmittances and wavelength range over which the instrument is going to be used. Ideally they should cover the full working range of the instrument. For wavelength ranges above 400 nm the best choice of neutral filters are glass filters because they do not have the possible added problem of interreflections that may be experienced when using metal-on-silica (MOS) filters. For wavelengths down to 200 nm, MOS filters are currently the only filters available. Typical spectra are shown in Figures 3 and 4.

Figure 3: Transmittances of glass neutral density filters**Figure 4: Transmittances of metal-on-silica neutral density filters**

5.4.2 Liquids

Liquids are an alternative to neutral density filters^[15]. However liquids usually only have a very limited useful range of wavelengths. They are also not neutral with wavelength as shown by the potassium dichromate spectrum in Figure 5. The resolution will vary with wavelength, and measurements are only recommended at minima, maxima or plateau regions.

Figure 5: Typical absorption spectrum of potassium dichromate solution



Solutions are not recommended for high accuracy work as some are prone to variation with pH, errors can be introduced in preparation e.g. in pipetting and weighing, and they have higher temperature coefficients than solid standards. However there are commercially available sealed solutions from a number of manufacturers.

5.4.3 Beers law (Bouguer-Lambert-Beer Law Test)

This only confirms that the detector response is a power function of the incident flux^[16]. Thus changing the concentration of the sample thickness (path length) only confirms that the detector follows the law but not how linear it is. Absorbance linearity should not be equated with transmittance accuracy. Beers law shows that the system follows a simple power law, not that the power is exactly unity.

5.5 Repeatability

Repeatability is assessed by measuring the random uncertainty under the same following measurement conditions: method of measurement, observer, measuring instrument, location and conditions of use. This is a Type A (evaluated by statistical methods) uncertainty^[17]. Repeatability data are obtained by measuring a sample, a number of times without moving the sample or changing any other condition of the measurement. It can be considered as a test of the measuring instrument's ability to repeat a measurement, and will always have a value smaller than for Reproducibility. The standard deviation of the measurements divided by the square root of the total number of measurements, then gives an indication of the measurement-to-measurement variation and can then be included in any total measurement uncertainty assessment.

5.6 Reproducibility

Reproducibility is also a Type A uncertainty ^[17]. It is evaluated by carrying out measurements at different times and where observer and conditions of measurement may have varied, within the acceptable ranges for the measurement. Reproducibility data can be obtained by measuring a sample, removing the sample, replacing the sample and making another measurement and then repeating this sequence a number of times. The replacement of the sample in the instrument needs to try and achieve the same alignment and measure the same area of the sample at the same angle as before. However it is essential to replace the sample each time as placement errors are a component of the reproducibility of measurement.

The standard deviation of the measurements divided by the square root of the total number of measurements then gives an indication of the measurement-to-measurement variation and can then be included in any total measurement uncertainty assessment.

5.7 Sample induced errors

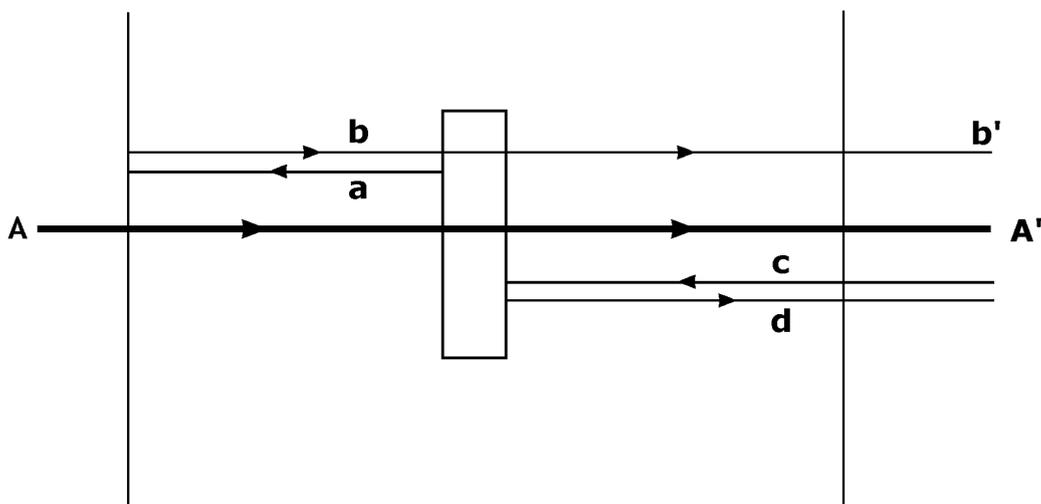
Some samples can contribute to errors. For example in focussed systems, samples with non-parallel sides can move and change size and path of the light patch, and hence the transmittance through the filter ^[12]. Transmittance through the filter is not constant across the wedge.

Calibrated traceable filters should not themselves contribute to such errors.

5.7.1 Interreflections

Interreflections between the sample and the detector or sample and illumination optics can occur in some spectrophotometers. This is especially a problem for metal-on-silica (MOS) filters ^[18].

Figure 6: Parasitic beams from interreflections off a MOS filter



In Figure 6 the main beam, A, is incident on a filter and A' is transmitted through the sample. Two parasitic beams are shown. Firstly the reflected beam, a, can be reflected by the instrument optics, b, and be transmitted as beam b', and secondly reflected beam, c, from the detector optics can be reflected by the filter as d. These result in a higher transmittance than the true value.

A filter that can be rotated in a holder that indicates angular position (Figure 7) is useful for assessing any interreflection problems when using this type or any other type of filter^[18, 19]. Some MOS filters also have absorption bands at approx 220 nm, 2200 nm and 2750 nm, due to water in the silica substrate.

Figure 7: Rotating filter for cuvette holder



If MOS filters are being used it is advisable to obtain a glass filter that is a match to one of the MOS filters at a wavelength in the visible spectrum. Both filters must be calibrated at this visible wavelength. For measurements made on the test spectrophotometer, if the glass filter gives the calibration value and the MOS filter does not, then this provides evidence of interreflection errors being present.

The problems of using MOS filters are well known, and research is being carried out to develop alternative filters for use at wavelengths below 400 nm.

5.7.2 Fluorescence

Some samples may fluoresce: they absorb light at one wavelength and emit light at another, usually longer wavelength. If the sample being measured is fluorescent then the two different configurations of light dispersion before or after the sample, (see 5.2), will almost certainly give different results. Either or both may give incorrect values, depending on the detection system and where in the spectrum the fluorescence occurs. The best way of measuring fluorescent samples for regular transmittance is to use a spectrofluorimeter that has monochromatic illumination and monochromatic detection that can be set independently ^[8].

5.8 Polarisation

Some materials may have transmittances that are polarisation dependant. The measured transmittance will also depend on the instrument's optical characteristics, e.g. the degree of collimation or convergence of the incident light, its polarization, and its degree of coherence.

Polarization is linear, elliptical or circular in nature and is generally wavelength-dependent. In most optical instruments, circular or elliptical polarization is negligible and the instrument polarization can be treated as primarily linear in nature. Linearly polarized light can be treated as a mixture of two components, a p- and an s-component, in which the vibration direction (electric field) of the incident radiation is respectively parallel or perpendicular to the plane of incidence. The plane of incidence is defined by the propagation direction of the radiation and the normal to the reflecting surface. If the instrument radiation comprises equal amounts of p- and s-polarized components, the instrument is regarded as unpolarized. If one of the components predominates, the instrument is partially polarized.

Regular transmittance measurements in a well-collimated instrument with the beam incident normally on the sample surface will be insensitive to instrument polarization. This will also hold for a convergent beam provided that the principal ray is normal to the sample and the vertical and horizontal angular spreads are equal, and have a cone angle of less than $\pm 5^\circ$.

Accurate determination of a sample's polarized transmittance components requires the use of a linear polarising element in the beam. The sample orientation is fixed and measurement carried out with the polarizer rotated to transmit p- and s-polarized light. It is important to note that in a double-beam instrument, the polarizer must be positioned in the common beam, i.e. before the beam is optically or mechanically split, or after it is recombined, to avoid beam-imbalance between the sample and reference beam. If this procedure is not followed, artefacts may appear in the ratioed spectra, particularly with grating instruments in the vicinity of Wood anomalies. If the sample is optically active, i.e. causes a rotation of the plane of polarization, a second linear polarizer and a quarterwave plate should be positioned after the sample to analyse the emergent beam.

An alternative approach is to accurately determine the degree of polarization of the instrument, and then carry out measurements with the sample in one orientation and then rotated by 90° about the beam axis ^[20, 21]. Fortunately, the polarization characteristics of most spectrophotometers are fairly stable and need only be redetermined every few years or whenever a particularly polarization-sensitive instrument component, such as a grating, is changed.

5.9 Wavelength scale

Wavelength scales are ideally assessed using narrow emission line discharge lamps. Wavelength lines for ultraviolet, visible and near infrared wavelengths can be obtained using Cd, Cs, He, Hg, Ne, Rb and Zn spectral lines^[22, 23]. For commercial instruments this is only possible if the instrument has an appropriate lamp built in or if an auxiliary lamp can be mounted in place of the source lamp. Some commercial instruments incorporate sources such as deuterium and mercury and often have an automated programme for wavelength calibration. However, these automated programmes close down the instrument slits to values much narrower than those normally used for absorption spectrophotometry.

The use of emission lamps requires that they be positioned to exactly reproduce the optical path obtained with the continuum lamp(s) employed in normal use. This together with the difficulty of uniformly irradiating the entrance slit of the spectrophotometer when used at large bandwidth settings can lead to calibration errors when using emission lamps^[24,25].

The alternative method is to use an absorbing wavelength standard which can be a solid^[6, 26, 27] or a liquid^[28] with narrow absorption bands, the peaks of which can be readily identified. Filters can be placed into the sample holder of the instrument. For practical purposes the wavelength of the absorption peaks are independent of temperature, although the actual transmittance at the peak may not be.

The wavelength scale of a spectrophotometer needs to be measured at many points as there may be non-linear differences across the scale due to stepper-motor linkages, lead screws, defects in the grating drive mechanism, positioning of array components, optics etc. Also components may wear unevenly. For instruments that produce coarse wavelength data at intervals greater than 0.5 nm, such as instruments containing diode arrays, wavelength peak positions can be found by curve fitting, e.g. a cubic spline, to the data points of a peak.

Wavelength repeatability can be measured in a similar way to repeatability of transmittance by placing a wavelength standard in the instrument and measuring to find the peak, and repeating this several times.

5.10 Stray light

Stray light is difficult to evaluate. It is light flux reaching the detector outside the spectral distribution prescribed for the measurement or which reaches the detector by a path other than that prescribed. Stray light can be both heterochromatic - of different wavelengths to those set by the spectral bandpass - and / or homochromatic - of the same wavelengths as those set by the spectral bandpass - but does not pass through the sample. The source of stray light can be internal or external.

External stray light is that from any source other than the instrument source. It is most likely to be found in instruments which are not adequately enclosed so that light may enter. The obvious way of checking for external stray light is to darken the room or cover suspect areas and note if there is any change in the output signal.

Internal stray light originates from the instrument source. Scattering due to diffraction occurs from system components such as gratings and lenses. Evaluation of heterochromatic stray light can be carried out with a series of long pass and bandpass filters^[29, 30]. Long-pass filters cut off short wavelengths and transmit at longer wavelengths, while bandpass filters transmit a small range of wavelengths. A series of these filters, which selectively block the wavelength of the incident light, can be placed in the measurement system to find the wavelengths associated with the stray light. Any signal detected is stray light. Care is needed when using cut-off filters as some may fluoresce. This can be a problem if the filter is between the monochromator and the detector.

There is a special filter that has a reflecting surface on one side and is opaque and blackened on the other. The reflecting surface should reflect a similar amount of light as samples. This is aligned with the reflecting surface facing the incident light and causes light to be back-reflected and scattered off the slits, around the sample compartment etc., but not through the sample position. The resulting measurement gives the isochromatic stray light from the source side of the filter. Turning this filter round to face the detector gives another component of isochromatic stray light.

Liquid solutions can also be used. Potassium iodide solution has been used for stray light evaluation in the UV below 260 nm.

5.11 Bandwidth

Monochromators transmit radiation of a finite bandwidth. For a particular grating the bandwidth can be varied by changing the width of the slits since these determine the extent of the dispersed spectrum. In practice some flux from outside the prescribed band will be transmitted due to the effects of optical aberrations, the wave nature of light and scattering. Bandwidth is usually defined as the full spectral width at half the maximum height (FWHM) and is usually not independent of wavelength although it is frequently assumed to be so.

The simplest method of measuring bandwidth is to use a highly monochromatic source and to monitor the variation of output with wavelength setting. This method is fairly straightforward with instruments such as reference spectrophotometers but may not be practicable with commercial instruments. At fixed slit-widths an emission line can be scanned and the bandwidth determined. For instruments with constant gain the bandwidth is continually changing and bandwidth cannot be easily measured.

5.12 Cleaning of standards

The best way of looking after solid reference standards is to make sure they are kept clean, by handling them as little as possible, wearing gloves and storing them in a closed box so they do not accumulate dust on them. It is best to use cotton gloves when handling unmounted filters. If they do become soiled they need to be cleaned to bring them back to their calibrated values.

There are different levels of cleaning that depend on the level of dirt associated with the standard^[31]. The lowest level of cleaning should be used first, with progression up a level if the standard still remains dirty. The simplest level is to use filtered (carbon and 22 μm paper) compressed air to remove dust and particles from the surface of the standard. The compressed air should be filtered to ensure that there is no oil in it, from the compression process, reaches the standard. If further light cleaning is required then breathing on it and using a clean cotton cloth to rub the surface is usually sufficient. For heavily soiled samples cotton wool pads soaked in ethanol or propanol should be wiped across the surface, followed while still wet with dilute detergent, e.g. decon 75, on a cotton wool pad. Rinsing in cold tap water should follow with observation of the evenness of surface wetting. If unsatisfactory then repeat the above using a more concentrated detergent solution. Follow with washing in warm running tap water for ten minutes, and then running distilled water for 1 minute, then dry using filtered compressed air. This can be summarised as mild techniques to start with, progressing to more reactive to remove heavy staining.

When using a liquid standard the cuvette used should be clean. Distilled water and ethanol is usually adequate to clean cuvettes for most applications, especially if the cuvette is emptied and cleaned straight after use. Care must also be taken to clean pipettes used to transfer solutions in and out of the cuvette.

5.13 Short and long term aging of spectrophotometer

Once a spectrophotometer has been assessed as passing the required specification and meeting the user's requirements steps need to be taken to ensure that it continues to meet them during use.

Simple checks should be carried out every day or every time the instrument is switched on. These can be as simple as checking the wavelength scale at a single wavelength and the transmittance / absorbance at one or two levels. Then on a longer time scale, e.g. weekly, depending on use and or regulatory requirements, more checks should be made. For example, check the wavelength scale at several wavelengths and transmittance / absorbance at several levels. A full check using all standards should be made periodically as well, e.g. monthly. This way a history of the performance of the instrument can be built up. This may aid in narrowing down the time of occurrence of a problem and in diagnosing the fault. In all cases if the instrument gives a measurement result outside the value plus or minus the uncertainty for the standard then the cause should be investigated, and a service engineer called if the instrument is at fault.

It is also useful to record environmental conditions at the time of measurement as they may influence the measurement result and cause anomalous effects, e.g. temperature of cell, sample compartment temperature, room temperature and relative humidity.

Terminology

6

Definitions for commonly occurring terms in spectrophotometry are given in this section.

Absorbance

Quantity expressing the absorption of radiation by a solution at a specific wavelength. This is a logarithmic measure calculated from the transmittance. Absorbance is linearly related to the path length and concentration of the solution. It is dimensionless but expressed in absorbance units (A):

$$A = \log_{10}(1/T) \text{ or } A = \log_{10}(100/T\%)$$

Absorption

The process by which radiation is attenuated on passing through a substance. The radiant energy is converted into some other form and is not scattered or refracted.

Cuvette / Cell

A container to hold solutions for the measurement of their absorption spectra. It should have two parallel optical windows fixed a specific distance apart.

Detector

The device in a spectrophotometer that measures the intensity of light.

Electromagnetic radiation / radiation

Wave motions of characteristic wavelength, including gamma radiation, X-rays, ultraviolet, visible light, infrared and radio waves.

Fluorescence

The emission of optical radiation when a substance is exposed to any electromagnetic radiation, where the emitted radiation generally appears within 10 nanoseconds after the excitation.

Infrared

The region of electromagnetic radiation of wavelengths from 800 nm to 1 mm. This can be divided into near infrared (800 nm to 2500 nm), mid infrared (2500 nm to 25 μm) and far infrared (25 μm to 1 mm).

Luminescence

The emission of optical radiation induced by any non-thermal process.

Optical Density

This is a general term for the absorbance of any material. Absorbance should only be applied to solutions. Optical density is most commonly used for optical materials such as filters, and is defined as:

$$D = \log_{10}(1/T) \text{ or } D = \log_{10}(100/T\%)$$

Phosphorescence

Any delayed emission of optical radiation, where the emitted radiation generally appears 10 nanoseconds or longer after the excitation.

Regular transmission

Transmission without deviation by scattering, diffraction or diffusion and with any photoluminescent components removed.

Regular transmittance

The ratio of the regularly transmitted optical radiation to the incident optical radiation under specified conditions of irradiance.

This results in a scale of 0 to 1 for transmittance. It is also common to express transmittance as a percentage.

$$T = I_T / I_0 \text{ or } T\% = I_T / I_0 \times 100$$

Where I_0 is the incident light and I_T the transmitted light.

Spectrophotometer

An instrument for measuring the transmittance or absorbance/optical density at different wavelengths.

Stray light

There are two types: a) heterochromatic – of different wavelengths to that set by the spectral bandpass of the measuring instrument and b) homochromatic – of the same wavelengths as spectral bandpass, but does not pass through the sample.

Transmission

The process by which optical radiation passes through a material.

Ultraviolet (UV)

The region of the electromagnetic spectrum at shorter wavelengths than blue light, generally taken to be between 100 nm and 400 nm. Due to atmospheric absorption UV measurements can only be carried out below 190 nm in a vacuum environment.

Visible

The region of the electromagnetic spectrum that can be seen by the human eye, generally taken to be between 400 nm and 800 nm.

Wavelength

The length of the waves associated with the electromagnetic radiation.

Reference Materials

7

IN THIS CHAPTER

- NPL supplied reference materials
 - » Neutral density filters
 - » Wavelength standards
 - » Transmittance colour standards
- Other reference materials

7.1 NPL Supplied reference materials

These are the materials that are routinely supplied, calibrated or recalibrated by NPL.

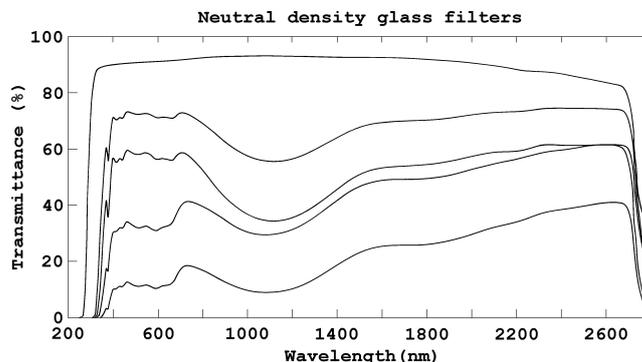
7.1.1 Neutral density transmittance standards

Figure 8: A set of glass neutral density filters



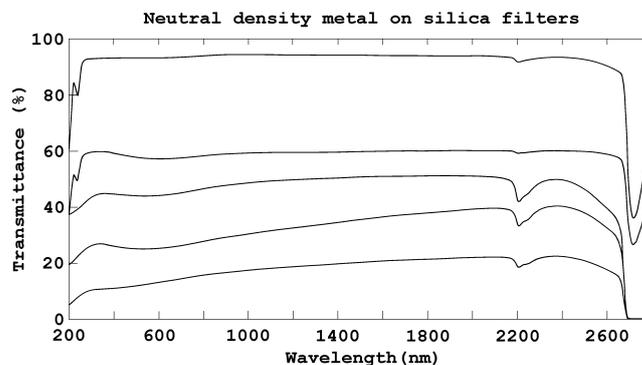
Neutral density transmittance standards are available for calibration in the wavelength range 200 nm – 2500 nm. There are two types: glass or metal on silica (MOS). Both types of neutral density filters are suitable in visible and near infrared (NIR) regions, but for UV measurements, MOS filters have to be used. The MOS filters are also more neutral than the glass filters, however, they are not as stable as glass and need to be calibrated more frequently. These MOS filters are useful for checking for inter-reflection effects. Filters can be supplied singly or in sets.

The filters supplied by NPL are mounted in frames approximately 56 mm x 12 mm x 12 mm in size, with a working aperture of 24 mm x 7 mm, that are designed to fit into 10 mm path length cuvette holders. Filters can be calibrated for regular transmittance $T\%$ or optical density (absorbance). Optical density, D , = $\log_{10} (100/T\%)$.

Figure 9: Spectra for glass neutral density filters

Glass filters are available in nominal optical densities of 0.05, 0.075, 0.13, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0. Calibration wavelengths are from 400 nm to 2500 nm.

MOS filters are available in nominal optical densities of 0.05, 0.25, 0.5, 1.0, 1.5 and 2.0. Calibration wavelengths are from 200 nm to 2500 nm.

Figure 10: Spectra for MOS neutral density filters

NPL is able to make regular transmittance measurements from 200 nm to 3000 nm using a Varian Cary 5/5E spectrophotometer. Spectral transmittance can be measured at either selected wavelengths or by using a scanning technique to measure at regular intervals between two wavelengths. Wavelength intervals are typically 5 nm but can be as low as 0.05 nm. The spectral bandwidth used for wavelengths up to 800 nm is chosen by the customer and can be up to 5.0 nm. Above 800 nm the bandwidth usually varies with wavelength up to a maximum of 20.0 nm. The bandwidth can be fixed for certain types of sample. NPL can also measure other sizes of neutral samples that are much smaller or larger in size. The temperature of measurements is usually 25°C. This is a UKAS accredited measurement service.

For regular transmittance between 350 nm and 800 nm typical uncertainties at the 95% confidence level, corresponding to a coverage factor $k=2$, are:

Table 1: Typical NPL regular transmittance uncertainties for UV-Visible wavelengths

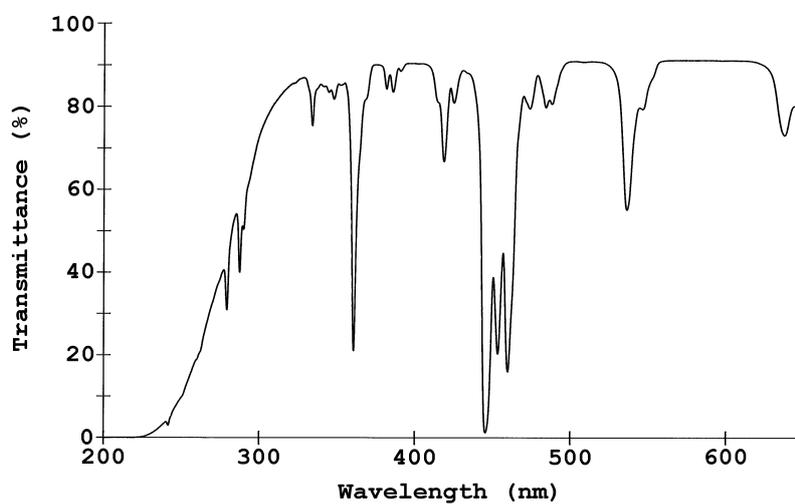
Transmittance T %	Uncertainty ΔT %	Density D	Uncertainty ΔD
90	± 0.20	0.046	± 0.0010
60	± 0.13	0.222	± 0.0009
30	± 0.10	0.523	± 0.0014
10	± 0.05	1.000	± 0.0022
1	± 0.007	2.000	± 0.0031
0.1	± 0.001	3.000	± 0.0037

The values listed in Table 1 assume that the accuracy of the measurement is limited by the measuring equipment and not the sample.

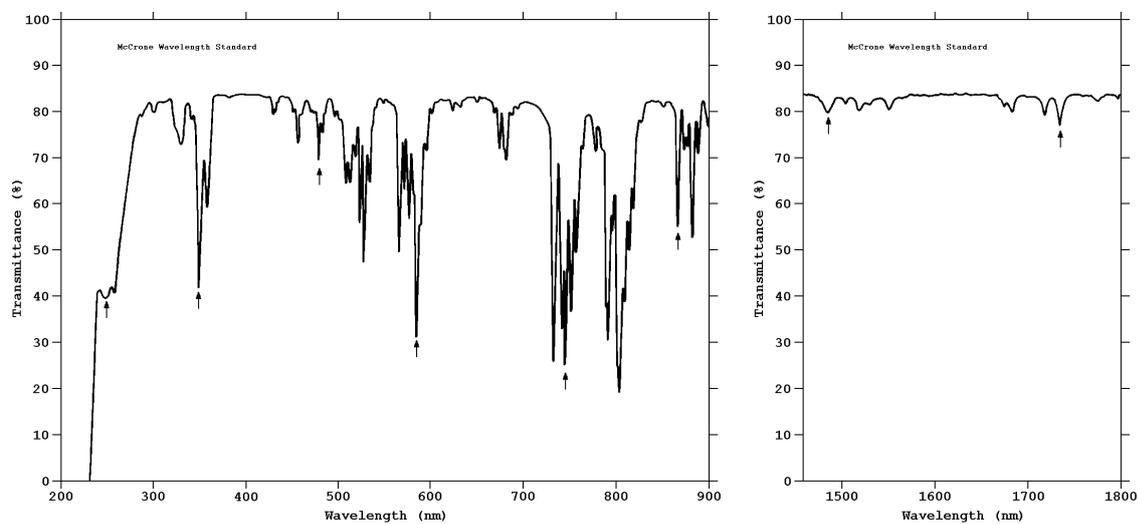
7.1.2 Wavelength standards

Several types of wavelength standard are available, McCrone, Nelson, Holmium Oxide, Russian S type and Russian E type. These standards have narrow absorption peaks and together cover the UV, Vis and NIR regions of the spectrum. The choice of wavelength standard will depend on spectral region of interest and bandwidth. The McCrone, Holmium and Nelson standards are mounted into frames approximately 56 mm x 12 mm x 12 mm in size. The working aperture for the McCrone and Holmium standards is 24 mm x 7 mm and for the Nelson wavelength standard is 15 mm x 7 mm. This is a UKAS accredited measurement service.

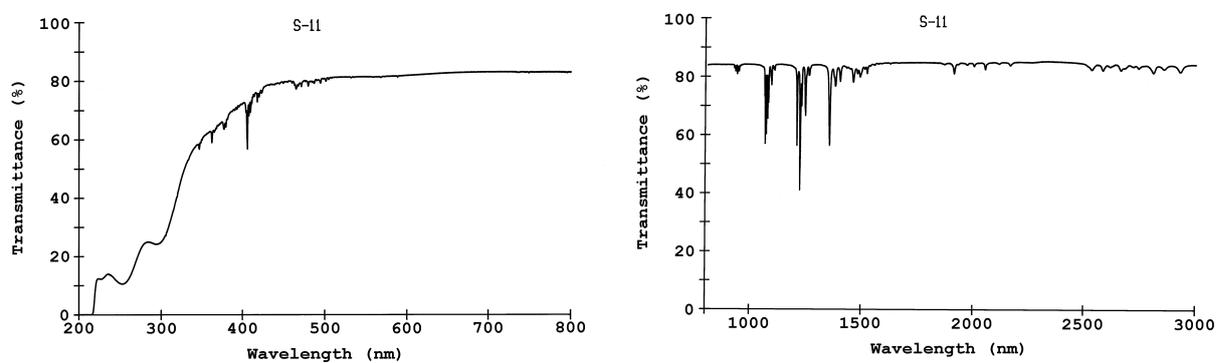
NPL can calibrate wavelength standards at the following nominal peak wavelengths and bandwidths. Other peaks may be possible for certain standards and bandwidths, contact NPL to discuss your requirements:

Figure 11: Holmium oxide wavelength standard spectrum**Table 2: Holmium oxide wavelength standard absorption peaks**

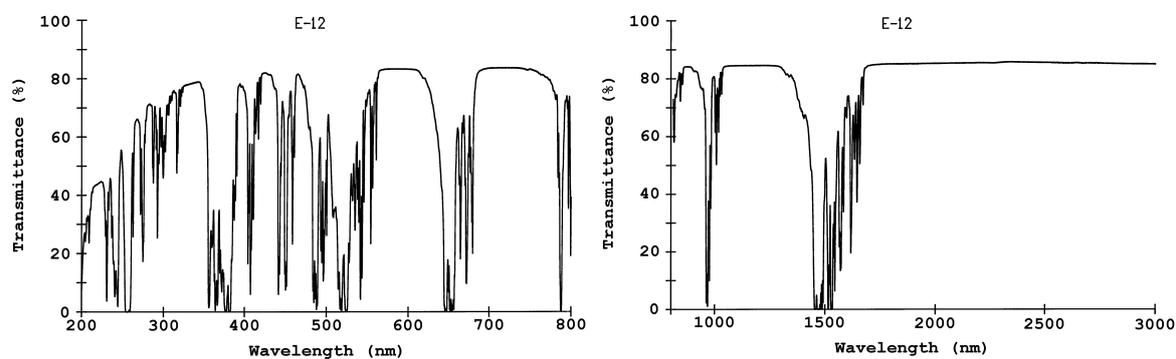
Wavelength of peak (nm)	Available calibration bandwidths (nm)		
241	0.5	1.0	
279	0.5	1.0	2.0
287	0.5	1.0	2.0
334	0.5	1.0	2.0
361	0.5	1.0	2.0
419	0.5	1.0	2.0
453	0.5	1.0	2.0
536	0.5	1.0	2.0
637	0.5	1.0	2.0

Figure 12: McCrone wavelength standard spectrum**Table 3: McCrone wavelength standard absorption peaks**

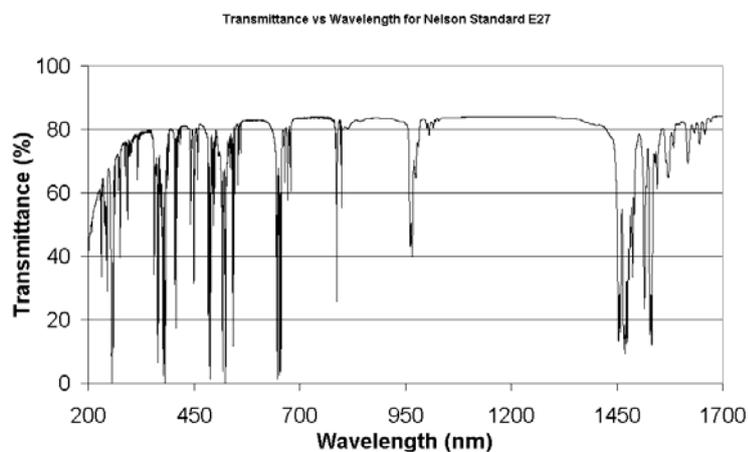
Wavelength of peak (nm)	Available calibration bandwidths (nm)			
	0.5	1.0	2.0	4.0
253	0.5	1.0		
354	0.5	1.0	2.0	
481	0.5	1.0	2.0	
589	0.5	1.0	2.0	
748	0.5	1.0	2.0	
869	0.5	1.0	2.0	
1485		1.0	2.0	4.0
1735		1.0	2.0	4.0
2327		1.0	2.0	4.0
2544		1.0	2.0	4.0

Figure 13: Russian S-type wavelength standard spectrum**Table 4: Russian S-type wavelength standard absorption peaks**

Wavelength of peak (nm)	Available calibration bandwidths (nm)		
1068	1.0	2.0	4.0
1211	1.0	2.0	4.0
1357	1.0	2.0	4.0

Figure 14: Russian E-type wavelength standard spectrum**Table 5: Russian E-type wavelength standard absorption peaks**

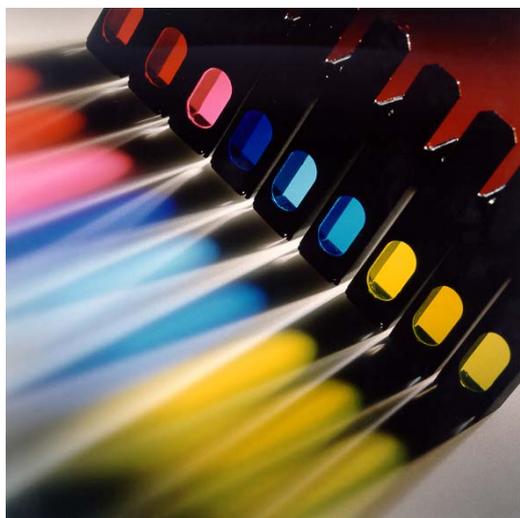
Wavelength of peak (nm)	Available calibration bandwidths (nm)		
209	0.5	1.0	2.0
231	0.5	1.0	2.0
263	0.5	1.0	
272	0.5	1.0	
293	0.5	1.0	2.0
316	0.5	1.0	2.0
1545	1.0	2.0	4.0
1645	1.0	2.0	4.0

Figure 15: Nelson wavelength standard spectrum**Table 6: Nelson wavelength standard absorption peaks**

Wavelength of peak (nm)	Available calibration bandwidths (nm)				Wavelength of peak (nm)	Available calibration bandwidths (nm)			
	0.5	1.0	2.0	4.0		0.5	1.0	2.0	4.0
209	0.5	1.0			555	0.5	1.0	2.0	
231	0.5	1.0	2.0		561	0.5	1.0	2.0	
244	0.5	1.0	2.0		664	0.5	1.0	2.0	
256	0.5	1.0	2.0		672	0.5	1.0	2.0	
275	0.5	1.0	2.0		788	0.5	1.0	2.0	4.0
293	0.5	1.0	2.0		797	0.5	1.0	2.0	
364	0.5	1.0	2.0		800	0.5	1.0	2.0	4.0
372	0.5	1.0	2.0		803	0.5	1.0	2.0	
404	0.5	1.0	2.0		1006		1.0	2.0	4.0
407	0.5	1.0	2.0		1475		1.0	2.0	4.0
459	0.5	1.0	2.0		1515		1.0	2.0	4.0
488	0.5	1.0	2.0		1545		1.0	2.0	4.0
524	0.5	1.0	2.0		1645		1.0	2.0	4.0
542	0.5	1.0	2.0						

7.1.3 Transmittance colour standards

Figure 16: NPL transmittance colour standards



NPL has available red, yellow and blue glass transmittance standards. For each colour there are 9 transmittance levels available, ranging from approximately 3% to 85%. The standards are 19 mm x 9 mm in size and are mounted in frames approximately 56 mm x 12 mm x 12 mm, with a working aperture of 15 mm x 7 mm, suitable for inserting in holders for 10 mm path length cuvettes. Measurement scans are made from 380 nm to 780 nm at 5 nm intervals and colorimetric data are calculated from the spectral data.

Colorimetric data such as, but not limited to, x , y , X , Y , Z , u' , v' , L^* , a^* , b^* can be calculated for CIE 2° and 10° Standard Observers and CIE Illuminants A, C, D50, D65.

7.2 Other reference materials

The range of other materials that can be used as standards is quite large and can be quite specialised. NPL is able to calibrate customers' own regular transmittance standards to their individual requirements.

Reference materials available include: neutral and coloured 50 mm square filters, larger area neutral and coloured filters, cut off and cut on filters, liquid absorbance standards, liquid wavelength standards, other solid wavelength standards, stray light filters, bandpass filters.

The sources for these reference materials include other national standards laboratories, other calibration laboratories, instrument manufacturers, optical supply companies, glass and filter manufacturers and specialist chemical companies.

Uncertainties in transmittance measurements

8

Example uncertainty budgets are given for wavelength standards and filters. These have been calculated according to ISO and UKAS guidelines ^[32, 33].

For a wavelength standard supplied by a standards laboratory there will be an uncertainty in the wavelength stated on the calibration certificate. Other uncertainties arise in the user's laboratory from the determination of the wavelength peak at the particular bandwidth and scan interval used to check the instrument. These uncertainties need to be combined together to give an overall uncertainty. The peak value from the certificate and the calculated overall uncertainty can then be used to determine if the measured peak is within the uncertainty range or not. If it is outside the uncertainty range then a wavelength error is present and either needs to be corrected for or the spectrophotometer adjusted to remove the error.

Table 7: Example uncertainty budget for a McCrone wavelength standard used to determine an instrument wavelength error at 589 nm

Symbol	Source of uncertainty	Value ± nm	Probability distribution	Divisor	c_i	$u_i(W)$ ± nm	v_i or v_{eff}
W_C	Certificate reference value	0.060	normal	2.00	1.0	0.030	∞
W_R	Repeatability	0.035	normal	2.00	1.0	0.018	5
W_{Re}	Reproducibility	0.030	rectangular	1.73	1.0	0.015	∞
$u_c(W)$	Combined standard uncertainty		normal			0.039	121
U	Expanded uncertainty		normal (k=2)			0.078	121

For a calibrated neutral density filter from a standards laboratory there will be an uncertainty in transmittance or density on the certificate. This needs to be used with the uncertainties for the user's spectrophotometer to determine the overall uncertainty at that transmittance for the instrument. A series of neutral filters covering the transmittances or densities of interest will give information on the photometric uncertainties. If any are outside the combined uncertainty range then a photometric scale error is present and can either be corrected for or the spectrophotometer adjusted to remove the error.

Table 8: Example uncertainty budget for a 10% MOS transmittance filter

Symbol	Source of uncertainty	Value ± T%	Probability distribution	Divisor	c_i	$u_i(T)$ ± nm	v_i or v_{eff}
T_R	Repeatability	0.0013	Normal	2.00	1.0	0.0007	5
T_M	Certificate Reference Value	0.0400	Normal	2.00	1.0	0.0200	∞
T_{Rd}	Reproducibility	0.0010	Rectangular	1.73	1.0	0.0006	∞
T_D	Drift over 12 months between calibrations	0.0430	Normal	2.00	1.0	0.0215	∞
T_T	Temperature coefficient for ± 1 °C	0.0013	Rectangular	1.73	1.0	0.0008	∞
$u_c(T)$	Combined standard uncertainty		Normal			0.029	20888775
U	Expanded uncertainty		normal (k=2)			0.059	20888775

Further Reading

9

For the reader who would like to enquire more into the aspects discussed in this guide the books listed below contain more detailed information.

Standards and Best Practice in Absorption Spectrometry, UV Spectrometry Group, Eds. C. Burgess and T. Frost, Blackwell Science Ltd., London (1999).

Spectrophotometry, Luminescence and Colour; Science and Compliance, (Analytical Spectroscopy Library Volume 6), UV Spectrometry Group/CORM, Eds. C. Burgess and D. G. Jones, Elsevier Science, Amsterdam (1995).

Advances in Standards and Methodology in Spectrophotometry, Eds. C. Burgess and K. D. Mielenz, Elsevier Science, The Netherlands (1987).

Practical Absorption Spectrometry, (Techniques in visible and ultraviolet spectrometry Volume 3), UV Spectrometry Group, Eds. A. Knowles and C. Burgess, Chapman and Hall, London (1984).

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Acknowledgements & Contact Details

11

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