

# Traceability for medical measuring devices through optical absorbance liquid filters

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**Abstract** – Many medical measuring devices (MMD), such as those used in clinical diagnostics, use optical absorbance as the measurand. Optical absorbance in MMDs is usually measured in microvolumes, according to pharmaceutical quality standards; some instruments should also be calibrated for absorbance at required specific wavelengths due to their construction. Optical absorbance liquid filters (OALF) are used to calibrate optical absorbance measurement equipment, but there is currently a capability gap across NMIs in the characterization and use of such filters, which leads to a lack of traceability.

Currently, precise calibration methods of MMDs for absorbance at required specific wavelengths are not possible using commercially available liquid traceable standards and the calibrations are performed at approximate wavelengths and absorbance levels. This paper describes the take up of traceable measurement capability of absorbance of liquid filters, the assessment of INRiM facility and the progress in the development of new OALF.

## I. INTRODUCTION

UV-Visible spectroscopy is one of the most widely used instrumental techniques in analytical chemistry, especially in the life sciences; it measures the amount of UV or visible light that is absorbed by a sample, to identify and quantify compounds in a variety of samples.

Spectroscopic analysis is commonly carried out in solutions. The Beer–Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, spectrum can be used to identify and to determine the concentration of the absorber in a solution. To obtain reliable spectral data, the absorbance scale of the spectrophotometer used for the measurements should be validated periodically against a reference material with certified absorbance at given wavelengths [1-3].

The reference materials suggested for the validation of a spectrophotometer on MMD, following the guidelines of the European Pharmacopoeia, are neutral density glass filters, for the visible range, and nicotinic acid solution (Niacin), for the UV range (with two characteristic peaks at 213 nm and 261 nm). The sealed quartz cuvettes of

potassium dichromate filters [4,5] widely used to check the absorbance scale in the UV range (characteristic wavelengths at 257 nm and 350 nm, close to the maxima, and characteristic wavelengths at 235 nm and 313 nm, close to the minima) are still permitted; the manual production of potassium dichromate solutions is no longer permitted because potassium dichromate is listed in Annex XIV of the REACH regulation. The liquid filters are used in UV range because glass filters do not transmit below 350 nm.

These reference materials are commercially available from accredited suppliers, therefore produced and calibrated following ISO 17034 [6] and/or ISO 17025 [7] standards. While regulators like the Pharmacopoeias may recommend certain reference materials, for validation of some MMDs operating, by construction, at specific wavelengths, any Certified Reference Material (CRM) with certified absorbance values at the required wavelengths may be used.

The overall objective of the ETraceAbs research project [8] funded by the European Union is to upgrade national metrological capacity of optical absorbance of liquid filters, to develop of new OALF covering the relevant wavelength used in MMD and to establish equivalence in the measurement of optical absorbance liquid filters among participating European NMI.

## II. MEASUREMENT FACILITY

INRiM maintains and operates the national scale of regular spectral transmittance. This calibration activity is part of the INRiM spectrophotometric measurement capability, which includes calibration for regular spectral transmittance, diffuse transmittance, regular and diffuse reflectance. The measurement facility provides calibrations of regular spectral transmittance for solid sample, such as optical filters.

The calibrations are performed with a commercial Agilent Cary 5000 UV-Vis double-beam spectrophotometer. This instrument can perform measurements in the wavelength range from 175 nm to 3300 nm with variable bandwidth from 5 nm down to 0.01 nm. In a double beam spectrophotometer the light from an halogen lamp (for the visible range) or a deuterium lamp

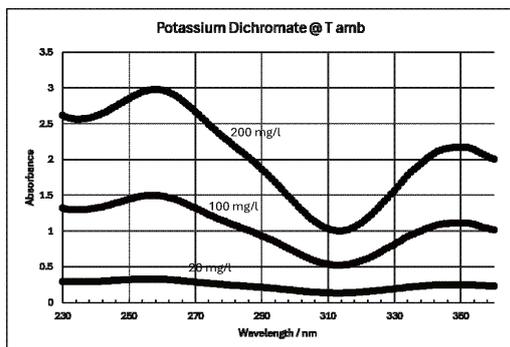


Fig. 1. Optical absorbance of potassium dichromate .

(for the UV range) is spectrally selected by a double monochromator and it is splitted into two beams: a reference beam and a beam that passes through the sample. Having two beams at the same wavelength allows simultaneous measurement of the reference and sample using individual detectors. The simultaneous readout of the detectors allows real-time compensation for any instrument fluctuations, resulting in stable measurements.

The facility has been recently upgraded with accessory to allows absorbance measurements of liquid in cuvettes and to control independently their temperatures, from 15 °C up to 40 °C.

### III. MEASUREMENT ASSESSMENT

Initial assessment of the optical absorbance measurement capacity of liquid filters has been performed using a commercial set of certified potassium dichromate sealed cuvettes with three different nominal concentration of 200 mg/l, 100 mg/l and 20 mg/l. Optical absorbance measurements (figure 1) have been performed at ambient temperature (23 °C) in the wavelength range from 230 nm to 380 nm, with 2 nm of spectral bandwidth. Preliminary measurement uncertainty estimations show fully compatibility with the values reported in the calibration certificate of the reference material nevertheless, doesn't yet meet the target uncertainty of 0,01 at 3,00 absorbance.

The following sections discuss the dependence of optical absorbance measurement on wavelength, bandwidth, temperature and the blank subtraction.

#### A. Wavelength and Spectral bandwidth

The wavelength corresponding to the maxima and minima of the absorbance spectrum were selected because they are the least subject to small deviations that may be present in the wavelength scale of the instruments for which the CRM is intended. The wavelength interval at which the spectrophotometer collects data points should be set to ensure that no spectral detail is lost but note that for measurement of filters with broad spectral bands, the

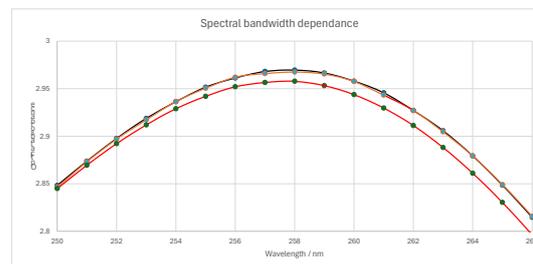


Fig. 2. Spectral bandwidth (SBW) dependence on 200 mg/l concentration of potassium dichromate; SBW = 5 nm red , SBW = 2 nm black and SBW = 0,5 nm orange

choice of this interval has little effect. A wider interval means fewer data points measured and therefore shorter measurement time.

The spectral bandwidth (SBW) is the spectrophotometer's ability to distinguish between two absorbance bands which are close together. Incorrect setting of SBW will result in loss of spectral detail and/or poor signal-to-noise; increasing resolution (smaller SBW) leads to increased spectral noise because less light is reaching the detector. Often the choice of SBW setting is a compromise, between resolution and signal-to-noise.

The example of the absorbance dependence on the spectral bandwidth for the 257 nm in potassium dichromate with 200 mg/l concentration is reported in figure 2. The measurements with 0,5 nm and 2 nm spectral bandwidth are practically overlapped (orange and black curves respectively) on top of the graph while the measurement with 5 nm spectral bandwidth (red curve) shows a significant reduction.

#### B. Temperature

Liquid standards can be affected by temperature, leading to changes in measured absorbance and/or peak shifts. Typically the CRM manufacturer specify the measurement temperature in the calibration certificate. Figure 3 shows the optical absorbance in a potassium dichromate filter with 200 mg/l concentration, close to the 257 nm absorbance peak wavelength, at three different temperatures, 15 °C (green curve), 23 °C (orange curve) and 37 °C (blue curve). The optical absorbance decrease with increasing temperature; the temperature coefficient at the certified wavelength is a few hundredths of a percent per Celsius.

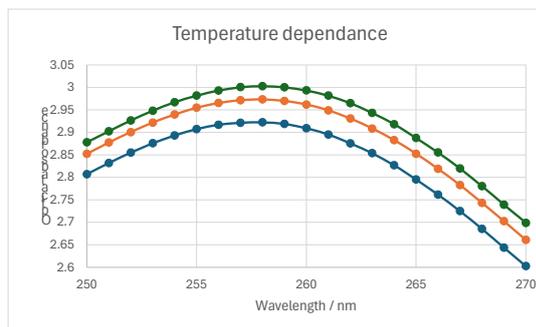


Fig. 3. Temperature dependence of potassium dichromate solution with 200 mg/l concentration: 15°C green curve, 23 °C orange curve and 37°C blue curve.

### C. Blank subtraction

To determine the specific absorbance of the liquid filter is necessary to subtract the absorbance of the cuvette with the blank solvent. This measurement plays a significant role as could be shown with the measurements of transmission losses in two high quality quartz cuvettes (figure 4). Main contribution to the transmission loss comes from the four reflections at the air-quartz interfaces; remaining contributions come from interreflections and imperfect parallelism of the windows.

## IV. CONCLUSIONS

This paper reports on the progress in the uptake of optical absorbance measurement in liquid filters, at INRiM spectrophotometric measurement facility.

Meanwhile, in the frame of ETraceAbs project, the other partners have identified with a survey the important wavelengths used in MMDs and have developed new liquid filters based on aqueous acid solutions of inorganic salts (potassium bichromate, chromium perchlorate, nickel sulphate, cobalt sulphate, copper sulphate). Selected liquid filters will be manufactured and shared among project partners to be independently characterized: homogeneity, pH, temporal stability and spectral measurements with spectral bandwidth and temperature dependence [9]. The results will be reported at the conference time.

## V. ACKNOWLEDGMENTS

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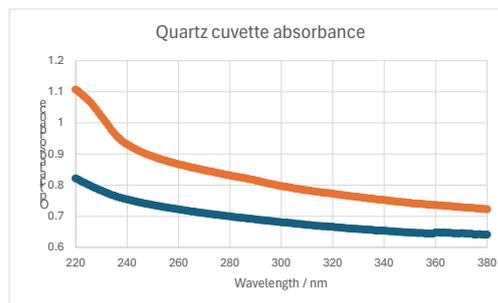


Fig. 4. Optical absorbance of two similar quartz cuvettes; blue curve shows a cuvette with absorbance close to the Fresnel losses.

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