Metrological characterization of standards – plastic films, which are used for calibration of nano volume spectrophotometers

Veljko Zarubica [1*], *Edvard Kubičela*[1], *Boban Zarkov*[2] and *Milesa Srećković*[3]

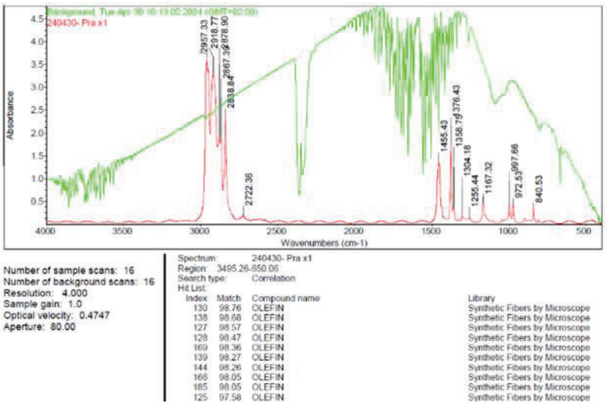
[1]Laboratory for calibration and validation, Analysis d.o.o, Japanska 4 Belgrade, Serbia
[2] Bureau of measures and precious metals, Mike Alasa 14 Belgrade, Serbia
[3]Faculty of Electrical Engineering, Belgrade,Serbia

Abstract. UV-VIS spectrophotometers, including nano drop (volume) spectrophotometers, provide an answer to all challenges in scientific and industrial laboratories, whether for pharmaceutical, chemical or service laboratories, all the way to more complex applications such as quantification of DNA, RNA, and protein per second, using only 1-2 µL of the sample. Certified reference materials are used to check the measurement capabilities of nanodrop spectrophotometers and other analytical instruments. Since these are solutions, which by themselves are not practical in terms of handling, stability, availability, we were thinking of using materials for their calibration, which would have more suitable properties than the solutions. We decided to use plastic films, which are thin enough not to be a problem during manipulation and measurement, which are stable in terms of determining the regular absorbance, which are optically neutral in the wider spectrum of optical radiation, and which are temperature stable and do not change their metrological characteristics during prolonged exposure to light. The paper evaluates all these influential characteristics and presents the budget of measurement uncertainty of plastic films in the UV part of the optical radiation spectrum at wavelengths of 260 nm and 280 nm (Fig.6). The term "metrologically characterized materials" represent equivalent of the materials, with precisely define regular absorbance (e.g., absorption), as well as estimated influential parameters which may appear, thus contributing to the uncertainty of the measurement.

1 INTRODUCTION

Calibration of Nano volume spectrophotometers is usually performed with some of the standard reference solutions. It is foreseen by the manufacturer of these instruments, that the mentioned solutions are applied for one-time use. After using these solutions, any excess solution is thrown away, which is basically the negative side of these SRMs. Of course, the other negative side of this method of calibration is the high price of the SRM. Our wish was to try to find a solution, namely SRM, such that the price of SRM is acceptable and that there is a possibility of using these SRM for a longer period. In this way, we would be able to calibrate Nano volume spectrophotometers with a completely different type of standard reference materials - foils. Our goal was to characterize these foils metrologically and present them as CRMs, which will be able to be used for a long period of time, without losing, or in the best case, degrading, their metrological properties, especially in terms of long-term stability, accuracy of reproducing the standard values etc. Before the characterization itself, the mentioned standard reference materials-foils were defined in terms of their composition analysis. We used an IR spectrophotometer with Fourier transform (FT-IR) to analyse the composition of the mentioned foils in detail.

Fig. 1. Spectral analysis by FT-IR plastic foils



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Number of sample scans:  16
Number of background scans:  16
Resolution:   4.000
Sample gain:  1.0
Optical velocity:  0.4747
Aperture:  80.00
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Spectrum:          240430- Pra x1
Region:   3495.26-650.06
Search type:       Correlation
Hit List:
  Index   Match   Compound name                              Library
   130    98.76   OLEFIN                                     Synthetic Fibers by Microscope
   138    98.68   OLEFIN                                     Synthetic Fibers by Microscope
   127    98.57   OLEFIN                                     Synthetic Fibers by Microscope
   128    98.47   OLEFIN                                     Synthetic Fibers by Microscope
   169    98.36   OLEFIN                                     Synthetic Fibers by Microscope
   139    98.27   OLEFIN                                     Synthetic Fibers by Microscope
   144    98.26   OLEFIN                                     Synthetic Fibers by Microscope
   166    98.05   OLEFIN                                     Synthetic Fibers by Microscope
   185    98.05   OLEFIN                                     Synthetic Fibers by Microscope
   125    97.58   OLEFIN                                     Synthetic Fibers by Microscope
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The results of the analysis are presented in Figure 1. It was important to determine the composition of the mentioned foils to see their hygroscopicity and resistance to UV radiation. It was found that they are olefins based on polypropylene, which represent materials with a poor hygroscopic characteristic and a very good characteristic of resistance to UV radiation, which is what we needed, because the key wavelengths for the application of these spectrophotometers were 260 nm and 280 nm. After analysing the foils, the metrological characterization of the foils was started:

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* Corresponding author: veljko.zarubica@analysis.rs

repeatability, homogeneity, exposure of the foils to sunlight and temperature influence.

2 Measurement Equipment and Methods

The measuring equipment in Laboratory for calibration and validation, Analysis d.o.o includes: sets of standard reference materials as standards of spectral transmittivity coefficients (Figure 2. One standard set with tree different foils).

First task is measuring uncertainty of optical plastic foils for absorbtion coefficient: 0,1A, 0,2A and 0,3A. We usually split the operating range in three ranges. The measuring run means 10 measuring points and comparison with certificates. We usually apply Xenon flash lamp. Various spectrometer types demand different procedures. To determine the metrological characteristics of optical plastic foils, a primary spectrophotometric system was used in the UV/VIS part of the spectrum of optical radiation from 190 nm to 1100 nm. The source of optical radiation is a xenon flash lamp, with a single monochromator and a double split beam. A semiconductor cell was used as a detector. The influencing parameters that occur during the measurement of the spectral coefficient of absorbance, i.e. absorption, of optically neutral filters are as follows:

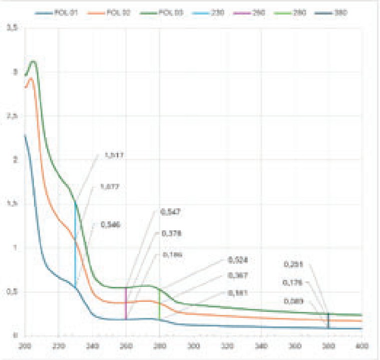
- homogeneity, exposure, temperature and atmospheric influences, long and short-term stability and the influence of dispersion.

In Laboratory Analysis d.o.o. a set of optically neutral foils type FOL1, FOL2 and FOL 3 of different values of spectral coefficient of absorption, were measured by placing a foil several micrometres thick on a plastic frame of appropriate dimensions of 25mm x 55mm.

2.1 Reproducibility determination of measurement of spectral coefficient absorption and wavelength setting

The determination of the repeatability of the measurement of the spectral coefficient of absorbance and the adjustment of the wavelength is carried out using filters of optical neutral foils in the UV and VIS areas of optical radiation.

Fig. 2. Absorption spectral characteristics foils type FOL



Reproducibility is performed in the UV part of the spectrum at wavelengths of 260 nm and 280 nm, while in the VIS part spectrum is performed at the beginning, middle and end of the VIS optical range. However, VIS optical range is not presented in this paper because the emphasis is in the UV part of the optical radiation. Which wavelengths we choose depends on the method used by spectrophotometer users. Since these are optically neutral foils, it is expected that at the mentioned wavelengths, the coefficient of spectral transparency is approximately the same value, nominally for foil (01) 0,20A, foil (02) 0,40A and foil (03) 0,60A. The spectral characteristics of our foils are shown in Fig 2.

Reproducibility is determined by repeating measurements after 24 and 48 hours, when talking about short-term stability, that is, measuring the foil over two years, when talking about long-term stability.

The paper was prepared during last two years, because the recalibration of plastic foils should be for one year, and in the worst case, 2 years.

The Fig. 3, 4 and 5 and table 1 show the absorption values of the filter foils obtained for monitoring over the course of one to two years.

Fig.3 FOL1

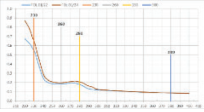


Fig.4 FOL2

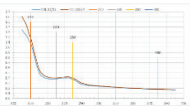


Fig.5 FOL3

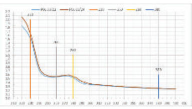


Table 1. Absorption values of the filter foils

Measuring the spectral coefficient of absorbance at a determined stability during a period of one year	FOL1		
	230 nm	260 nm	280 nm
Average value of spectral absorbance coefficient in 2022	0,5476	0,1833	0,1787
Average value of spectral absorbance coefficient in 2024	0,6505	0,1968	0,2052
Δ Absolute deviation	0,1029	0,0135	0,0264
Relative deviation of measurements (%)	18,79	7,39	14,79
Measuring the spectral coefficient of absorbance at a determined stability during a period of one year	FOL2		
	230 nm	260 nm	280 nm
Average value of spectral absorbance coefficient in 2022	1,0844	0,3848	0,3725
Average value of spectral absorbance coefficient in 2024	1,1980	0,4004	0,3934
Δ Absolute deviation	0,1136	0,0156	0,0209
Relative deviation of measurements (%)	10,47	4,05	5,60
Measuring the spectral coefficient of absorbance at a determined stability during a period of one year	FOL3		
	230 nm	260 nm	280 nm
Average value of spectral absorbance coefficient in 2022	1,5208	0,5489	0,5267
Average value of spectral absorbance coefficient in 2024	1,6415	0,5586	0,5594
Δ Absolute deviation	0,1208	0,0097	0,0326
Relative deviation of measurements (%)	7,94	1,77	6,19

Based on the results obtained over a period of 2 years, depending on which wavelength we are talking about, it can be seen that the relative deviation of the measured absorbance values differs significantly between foils, and also the absorbance values for the same foil during a time period of 2 years.

Monitoring these foils for a period of less than a year gave much better results, showing that if these foils were to be used as a working standard, their calibration would have to be done for at least a year. We will show that in the further text of this paper. On Fig. 6 to 8 show the spectral characteristics of the absorption coefficient for three different filter foils, as well as the absorption values at precisely determined wavelength values monitored over the course of one year. Values are much better, which can be seen from Table 2. The RSD values, ie the standard deviations of the results in a period of one year, are significantly better.

Fig. 6. FOL 1

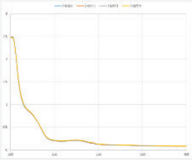


Fig. 7. FOL 2

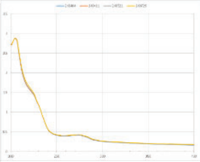


Fig. 8. FOL 3

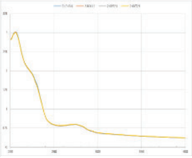


Table 2. Stability of coefficient absorbance during one year

Measuring the spectral coefficient of absorbance at a determined stability during a period of one year	FOL1		
	230 nm	260 nm	280 nm
240104	0,2275	0,5425	0,5323
240311	0,2244	0,5383	0,5275
240521	0,2204	0,5371	0,5226
240926	0,2277	0,5267	0,5194
241129	0,2233	0,5353	0,5137
Average τ	0,2247	0,0049	0,5231
u component of measurement uncertainty of stability	0,0070	0,0050	0,0059
u_{ave}	0,0104		
Measuring the spectral coefficient of absorbance at a determined stability during a period of one year	FOL2		
	230 nm	260 nm	280 nm
240104	0,0640	0,4018	0,4085
240311	0,0641	0,4017	0,4087
240521	0,0632	0,3990	0,4048
240926	0,0656	0,3999	0,3999
241129	0,0652	0,3924	0,3987
Average τ	0,0644	0,3989	0,4041
u component of measurement uncertainty of stability	0,0078	0,0050	0,0060
u_{ave}	0,0110		
Measuring the spectral coefficient of absorbance at a determined stability during a period of one year	FOL3		
	230 nm	260 nm	280 nm
240104	0,0215	0,2766	0,2745
240311	0,0229	0,2785	0,2782
240521	0,0230	0,2787	0,2784
240926	0,0223	0,2722	0,2710
241129	0,0228	0,2707	0,2709
Average τ	0,0225	0,2754	0,2746
u component of measurement uncertainty of stability	0,0142	0,0069	0,0069
u_{ave}	0,0173		

2.2 Determination of foil homogenity

The homogeneity test of optically neutral films is based on the method of determining the spectral absorbance of films at different positions, i.e. different points on the active surface of the film. Since these are relatively small dimensions of the active surfaces of the foils, the homogeneity test is performed in small increments of a few mm to the left, right, up and down in relation to the central point of the foil, with a beam diameter of 1 to 2 mm. In this way, the best coverage of the active surface of the foil is achieved. The number of measuring points, of course, will depend on the size of the active surface, which in our case amounted to 5 measuring positions. The illumination of the measuring foil was achieved by the method shown in Figure 9. The integration sphere evenly illuminates the filter, where a part of the transmitted optical radiation passes through the telescope and reaches the photocell. The telescope is positioned to define the measuring field. In Figure 9. an optical diagram of the measurement method for checking the homogeneity of the filter is presented. The telescope consists of a collecting lens, an aperture mirror, a colorimetric lens and an eyepiece, which can be used to more easily define the measuring field on the filter. A collimator lens is needed to form a uniform light beam to illuminate the photocell evenly. An aperture and an aperture mirror are needed to define the measuring field. From the photocell, the signal is routed to a voltmeter via a transimpedance amplifier. The filter, which is located directly at the exit of the integration sphere, is located on a movable support with a scale applied. The carrier can be driven manually along the x,y axis with the possibility of fine translation in steps of 1 mm. In this way, it is possible to test the homogeneity of the filter at different points. All measurements were performed in the Directorate of Measures and Precious Metals.

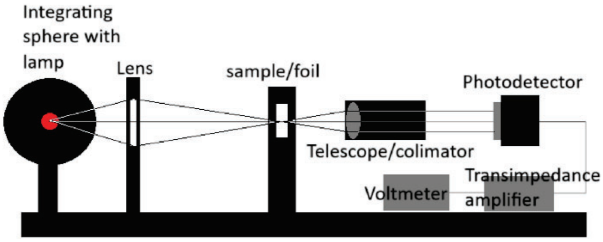


Fig. 9. Schematic representation of the method for verifying the homogeneity of filters of spectral coefficient of absorbance standards.

Table.3 Measurement uncertainty, resulting from inhomogenity of the three spectrophotometric filter fols

Measuring of the spectral coefficient absorbance on different position on the foil surface	FOL1	FOL2	FOL3
Mean valus of 5 different postions	**0,2270**	**0,6403**	**0,6306**
u – component of measurement uncertainty due to inhomogenity surface of foils	0,00006	0,0019	0,0025
U_{ave}	0,0032		

Based on the measured values of the spectral coefficient of absorbance at each point of the active surface for each film, the values of the measurement uncertainty of the measurement, which originate from the inhomogeneity of the filter, were obtained. Table 3 presents the calculated values of the measurement uncertainties for all three spectrophotometric optically neutral films separately, as well as the overall measurement uncertainty.

2.3 Determination of exposure of light radiation influence

The method of determining the influence parameter resulting from short-term exposure of spectrophotometric filters to optical radiation consisted in continuous exposure of all three spectrophotometric optically neutral foil filters to radiation in a period of 24 hours, with measurements of the spectral coefficient of absorbance performed before and after exposure. The measurements were performed on the primary spectrophotometric system at three different wavelengths of 230nm, 260nm and 280nm (Tables 4. and 5.). The reference conditions under which the measurements were carried out are as follows:

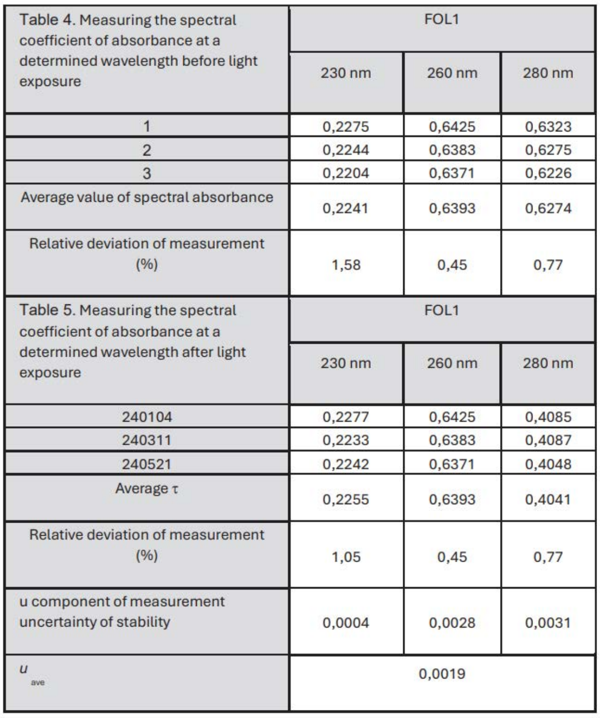


Table 4. Measuring the spectral coefficient of absorbance at a determined wavelength before light exposure	FOL1		
	230 nm	260 nm	280 nm
1	0,2275	0,6425	0,6323
2	0,2244	0,6383	0,6275
3	0,2204	0,6371	0,6226
Average value of spectral absorbance	0,2241	0,6393	0,6274
Relative deviation of measurement (%)	1,58	0,45	0,77

Table 5. Measuring the spectral coefficient of absorbance at a determined wavelength after light exposure	FOL1		
	230 nm	260 nm	280 nm
240104	0,2277	0,6425	0,4085
240311	0,2233	0,6383	0,4087
240521	0,2242	0,6371	0,4048
Average τ	0,2255	0,6393	0,4041
Relative deviation of measurement (%)	1,05	0,45	0,77
u component of measurement uncertainty of stability	0,0004	0,0028	0,0031
u_{ave}	0,0019		

u_{ave}- The mean value of the measurement uncertainty, which arises from the exposure of light for a given set of foils

2.4 Determination of temperature influence

The method of determining the temperature effects on spectrophotometric filters-films is performed using the primary spectrophotometric system and consists in measuring the spectral coefficient of absorbance of standard-spectrophotometric films at a room temperature of 20.5 ºC and at a temperature of 7.5 ºC at several different values of wavelengths of 230 nm, 260 nm and 280 nm.

The measurement was performed on a set of three foils FOL1, FOL2 and FOL3 with nominal values of spectral absorbance coefficient of (01) 0,20A, foil (02) 0,40A and foil (03) 0.60A.

The main problem of this method was to maintain the temperature within the given limits. The method consisted in determining the values of the spectral coefficient of absorbance at a temperature of 20.5ºC before cooling the standard foils at a temperature of 7.5°C, and then comparing the values obtained. Why did they go for such a temperature interval? Primarily due to the possible impact of condensation formation or dew of the standard foil, which would lead to an error in reading the value of the spectral absorbance coefficient. It has been shown that the foils have quite satisfactory characteristics in terms of temperature influences. Very small deviations of measurement uncertainties at different temperatures were obtained, which are shown in Tables 6 and 7. The difference was only 0.00017 of the spectral absorbance coefficient.

Table 6. Measuring on 20,5 ºC

Measuring results spectral coefficient absorbance on 20,5 ºC	**FOL 1**	**FOL 2**	**FOL 3**
Mean value of series at 6 measuring	**0,2255**	**0,6288**	**0,6155**
Standard deviation	0,00004	0,00007	0,00003
RSD %	0,00016	0,00011	0,00005
Measurement uncertainty	0,00009	0,00006	0,00003
Uav	0,00011		

Table 7. Measuring on 7,5 ºC

Measuring results spectral coefficient absorbance on 7,5 ºC	**FOL 1**	**FOL 2**	**FOL 3**
Mean value of series at 6 measuring	**0,2222**	**0,6322**	**0,6161**
Standard deviation	0,00010	0,00011	0,00007
RSD %	0,00044	0,00017	0,00012
Measurement uncertainty	0,00025	0,00010	0,00007
Uav	0,00028		

2.5 The Influence of all components measuring uncertainty of the spectral absorbance of standard filter-foil (budget of measurement uncertainty)

Based on the measurements of all the influencing parameters due to the determination of the metrological characteristics of the standard filter films, we have obtained an overall assessment of the measurement uncertainty of the filter films themselves. The individual components of the measurement uncertainties are presented in Tables 8, 9 and 10, which also represents the budget measurement uncertainty of the spectral coefficient absorbance for each filter foil.

Tables 8, 9 and 10 show budget measurement uncertainty of the spectral absorbance coefficient for each filter foil

Table 8. The source of measurement uncertainty	FOL1	
	Contribution of standard uncertainty (Type A)	Contribution of standard uncertainty (Type B)
Component of measurement uncertainty of spectrophotometric system (dispersion of results, drift, stray light, resolution, fault radiation, setting wavelength…)		0,00104
Long stability of the results of measurement of spectral coefficient of absorbance after one year	0,0104	
Component of measurement uncertainty of homogeneity of filter structure	0,0006	
Component of measurement uncertainty of exposure of light radiation	0,0004	
Component of measurement uncertainty of temperature influence	0,0017	
Standard uncertainty by measurement of spectral absorbance coefficient	0,0106	0,00104
Combined uncertainty of the measurements of the absorbance coefficient with k=2	0,0213	

Table 9. The source of measurement uncertainty	FOL2	
	Contribution of standard uncertainty (Type A)	Contribution of standard uncertainty (Type B)
Component of measurement uncertainty of spectrophotometric system (dispersion of results, drift, stray light, resolution, fault radiation, setting wavelength…)		0,00104
Long stability of the results of measurement of spectral coefficient of absorbance after one year	0,0110	
Component of measurement uncertainty of homogeneity of filter structure	0,0019	
Component of measurement uncertainty of exposure of light radiation	0,0028	
Component of measurement uncertainty of temperature influence	0,0002	
Standard uncertainty by measurement of spectral absorbance coefficient	0,0115	0,00104
Combined uncertainty of the measurements of the absorbance coefficient with k=2	0,0231	

Table 10. The source of measurement uncertainty	FOL3	
	Contribution of standard uncertainty (Type A)	Contribution of standard uncertainty (Type B)
Component of measurement uncertainty of spectrophotometric system (dispersion of results, drift, stray light, resolution, fault radiation, setting wavelength…)		0,00104
Long stability of the results of measurement of spectral coefficient of absorbance after one year	0,0173	
Component of measurement uncertainty of homogeneity of filter structure	0,0025	
Component of measurement uncertainty of exposure of light radiation	0,0031	
Component of measurement uncertainty of temperature influence	0,0001	
Standard uncertainty by measurement of spectral absorbance coefficient	0,0177	0,00104
Combined uncertainty of the measurements of the absorbance coefficient with k=2	0,0356	

3 Conclusion

The purpose and intention of this paper was to expand the scope of work of accredited laboratories in the field of spectrophotometric measurements and to follow the modern tendency of nano measurements using instruments that provide this possibility - nano drop spectrophotometers. The idea was to implement standard filters, which would be a means of transferring values from higher hierarchical levels of spectrophotometric measurements to lower levels, i.e. to nano drop spectrophotometers, and thus carry out metrological traceability to the national standard. In addition to many materials, the choice fell on foils, which perfectly match the measurement geometry of nanodrop spectrophotometers and can be characterized relatively easily by metrology. This is how the process of metrological characterization of standard filter foils began, which, after the realization of this project, began to be a means of transmitting the values of the spectral coefficient of absorbance, i.e. absorption. In this way, we have realized a new type of spectrophotometric filters, which will be used for the calibration of nano drop spectrophotometers. At the same time, we have methodologically simplified the process of calibration of nanodrop spectrophotometers, and also reduced the cost of the calibration process. The filter film standards themselves have proven to be good standards in terms of metrological characteristics, both in the short and long term. The aim of this paper was primarily to create a new, cheaper and sufficiently reliable tool for the calibration of nano drop spectrophotometers. In order to achieve this, it was necessary to determine all relevant metrological parameters in terms of metrological characterization of the given standard-filter films, i.e. setting up methods for the characterization of filter films, measurement, analysis of the results obtained, as well as the estimation of the calculation of the measurement uncertainty of filter films. In this way, the story of realization of standard sets obtained from plastic foils is wrapped up.

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