Spectrophotometer standardization in UV-Vis spectral region

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Abstract: Ultraviolet-visible (UV-Vis) spectrophotometry is commonly used in analytical laboratories for qualitative and quantitative analyses. To make the data obtained reliable, an experimental instrument must be calibrated. National Institute for Standards (NIS) in Egypt builds up a photometric method to ensure the competence of absorbance and transmittance to the standard international and national requirements. This paper presents an instrument for UV-Vis measurement, discusses the factors affecting measurement reliability and estabishes the uncertainty model including correspoding measurement parameters.

Key words: spectrophotometer; standardization; absorbance; uncertainty

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The spectrophotometer is a very powerful tool for high throughput routine testing for a comprehensive variety of analysts in chemical and biochemical analysis laboratories. The popularity of ultraviolet-visible (UV/Vis) spectrophotometry technique stems from its ease of use and the speed of the analysis[1]. It is important for accuracy and reproducibility in measurement that this instrument is correctly setup and calibrated to provide traceability to absorbance or transmittance[2-3]. The absorbance data from UV-Vis measurement can be related to the concentration of the sample by Beer's Law which describes the linear relationship between absorbance and concentration[4].

The basic principle of UV/Vis spectrophotometry depends on that light is generated by a source lamp which is normally a tungsten lamp for the visible region of the spectrum and deuterium for the ultraviolet range. The light is dispersed into its constituent wavelengths in a monochromator which results in a narrowband of the dispersed spectrum passing from the exit slit of the monochromator. Suitable optics is used to lead this light, of a narrow wavelength band, to the sample to be measured. Fig. 1 is a schematic of a dual-beam spectrophotometer[5].

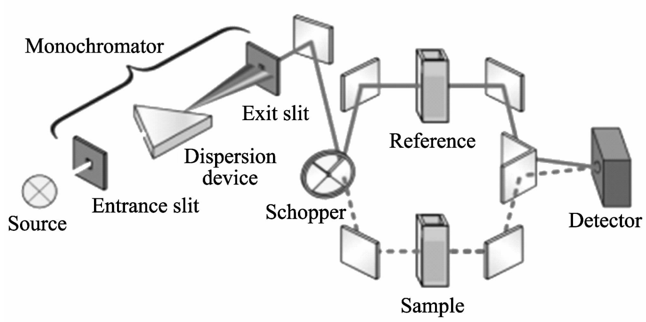

Fig. 1 Optical system of a dual-beam spectrophotometer

1 Main research work

UV-Vis spectrophotometer with slit width 1 nm, type UV-3101 PC from Shimadzu Inc, is used. It has dual light sources. A deuterium lamp is used for UV range and a tungsten lamp is for visible range. It has a double-monochromator system using two sets of three holographic gratings and different blazed wavelengths for use at maximum efficiency region, which provides a wide wavelength measurement from 190 to 3 100 nm. Gratings are automatically selected to provide the highest efficiency according to the measuring wavelength range. Its resolution is 0.1 nm and the stray light is less than 0.000 1%. The UV-3101 PC spectrophotometer is communicated with an external

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computer, which permits a full operation of the instrument through the keyboard[6]. The factors affecting reliability of UV-Vis measurement will be discussed. The required performance mainly includes the calibration of wavelength scale and photometric scale (expressed on absorbance) other factors such as spectral bandwidth, baseline flatness, resolution, and stability will also be discussed.

2 Results and discussion

2.1 Spectral bandwidth

Although a narrower spectral bandwidth improves the resolution of closely spaced peaks, it also decreases signal-to-noise ratio[7-8]. thus a spectral bandwidth should be chosen based on the needs of the analysis being performed. To avoid errors due to spectral slit-width, when using UV-3101 on which the slit-width is variable at the selected wavelength, the slit-width must be small compared with the half-width of the absorption band, but it must be as large as possible to obtain a high intensity value. The spectral bandwidth of the instrument will always be narrower than the spectral slit width. Therefore, a slit width is chosen such that further reduction does not result in a change in absorbance, 1 nm here.

2.2 Baseline flatness

The intensity of the radiation coming from the light sources is not constant over the whole UV-Vis range. The response of the detector also varies over the spectral range. A flat baseline demonstrates the ability of the instrument to normalize the output of the lamp and detector responses. This flat baseline is made at each established standard value; either in wavelength or photometric (absorbance) scale.

2.3 Calibration of absorbance scale

The absorbance of a solution is a logarithmic ratio of the radiation falling upon a material (I_0), to the radiation transmitted through a material (I) and this equal to the logarithm to base 10 of the reciprocal of the transmittance (T) for monochromatic radiation.

Absorbance at a certain wavelength of light (λ), denoted A_λ, is a quantitative measure expressed as[4]

$$A_\lambda = \log_{10}\left(\frac{I_0}{I}\right) = \log_{10}\left(\frac{1}{T}\right). \quad (1)$$

The most accurate method of calibrating the absorbance scale is to use Neutral Density (ND) glass filters designed to give a specified absorbance/transmittance value at a specified wavelength. For the control of absorbance, use a set of six neutral density glass filters manufactured in Starna Scientific Ltd. with nominal absorbance values: 0.04, 0.1, 0.3, 0.5, 1 and 2 arbitrary unit (AU) calibrated at specified five wavelengths are used at 1 nm spectral band width[9]. The spectrophotometer absorbance error (A_{error}) on these measurements is determined by calculating the difference between the certified filter absorbance and average measured absorbance of repeating five times. The spectrophotometer absorbance error results are given in Table 1.

Table 1 Results of photometric scale (absorbance)

ND-filter (Nominal absorbance)	Wavelength (nm)	Avg. measured absorbance (AU)	Absolute error (Avg. - certified) (AU)	Expanded uncertainty (k=2) (AU)
0.04	440	0.042	0.001	±0.006
	465	0.040	0.000	
	546.1	0.040	0.001	
	590	0.039	0.000	
	635	0.039	0.001	
0.1	440	0.148	−0.002	±0.006
	465	0.134	−0.002	
	546.1	0.134	−0.002	
	590	0.141	−0.003	
	635	0.143	−0.002	
0.3	440	0.339	−0.002	±0.006
	465	0.301	−0.002	
	546.1	0.303	−0.002	
	590	0.324	−0.002	
	635	0.329	−0.002	
0.5	440	0.556	0.003	±0.006
	465	0.505	0.002	
	546.1	0.517	0.001	
	590	0.545	0.000	
	635	0.530	0.000	
1	440	1.066	0.004	±0.006
	465	0.981	0.002	
	546.1	0.993	0.000	
	590	1.035	−0.001	
	635	0.988	−0.001	
2	440	2.137	0.000	±0.012
	465	1.990	0.002	
	546.1	2.010	0.001	
	590	2.047	−0.002	
	635	1.938	0.000	

2.4 Calibration of wavelength scale

The ideal wavelength standard would have very narrow and defined peaks.

Two different filters, Holmium and Didymium glass filters, manufactured in Starna Scientific Ltd, are used for calibration of the wavelength scale. Both filters are certified at specified eleven wavelengths of peak maxima as referenced to air[8-9].

The monochromator wavelength error (λ_{error}) on these measurements is done by calculating the difference between the certified peak maxima and average measured peaks maxima of repeating five times.

Figs. 2 and 3 show all revealed peaks, of Holmium and Didymium glass filters for one of five repeat absorbance measurements.

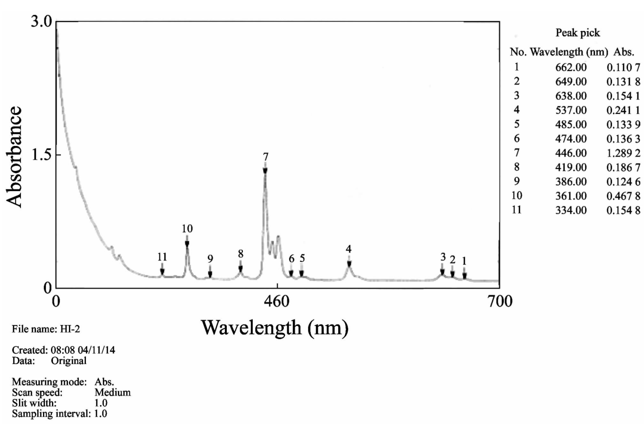

Fig. 2 Peaks of Holmium glass filter for one absorbance measurement

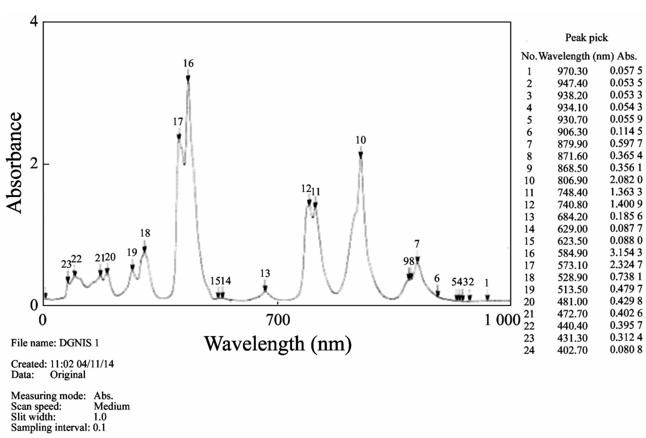

Fig. 3 Peaks of Didymium glass filter for one absorbance measurement

Tables 2 shows the results of wavelength scale error using Holmium glass filter.

Table 2 Wavelength scale results using Holmium filter

Filter identification	Avg. measured peak (nm)	Absolute error (nm) (Avg. - certified)	Expanded uncertainty (nm) (k=2)
Holmium glass filter	241.5	0.1	±0.4
	279.3	0.2	
	287.5	0.1	
	333.8	0.1	
	360.9	0.1	
	418.8	0.1	
	445.7	0.0	
	453.7	0.0	
	460.2	0.0	
	536.4	−0.1	
	637.5	−0.2	

2.5 Uncertainty analysis

The associated uncertainty must be quoted whenever measurement results are reported. Uncertainty analysis is a fundamental part of metrology. uncertainty evaluation is done by the guide to express uncertainty in measurement (GUM) method described by International Organization for Standardization (ISO) [10]. The standard uncertainty $u(x_i)$ to be associated with input quantity x_i is the estimated standard deviation of the mean[10-11],

$$u(x_i) = s(\overline{X}_i) =$$

$$\left(\frac{1}{n(n-1)}\sum_{k=1}^{n}(X_{i,k}-\overline{X}_i)^2\right)^{1/2}. \quad (2)$$

The combined standard uncertainty $u_c(y)$ is obtained by combining individual standard uncertainties u_i, evaluated as Types A and B

$$u_c^2(y) = \sum_{i=1}^{N}\left(\frac{\partial f}{\partial x_i}\right)^2 u^2(x_i). \quad (3)$$

Expanded uncertainty is calculated according to the following model

$$U_{\text{exp}} = 2U_C = 2\sqrt{(U_A)^2+(U_B)^2}, \quad (4)$$

where U_{filter} is uncertainty due to reference standard filters at $k=2$; U_{drift} is uncertainty due to drift of the standard, estimated from annual calibrations; $U_{\text{resolution}}$ is uncertainty due to resolution of the spectrophotometer and $U_{\text{straylight}}$ is uncertainty due to stray light of the spectrophotometer.

3 Conclusion

The objective of this paper is to evaluate and standardize the UV-Vis region performance of NIS institute spectrophotometer, type UV-3101 PC from Shimadzu Inc. The absolute error in the wavelength and photometric (absorbance) scale is calculated. The accompanied expanded uncertainty with the measurement is maximally ±0.012 nm in the absorbance scale and ± 0.4 nm in the wavelength scale. Although these procedures need not be performed every time the spectrophotometer is used, they should be performed on a routine basis to keep track of instrument performance over time or whenever data quality appears to be degraded. In conclusion, many performance verification tests have to be done on a UV-Vis spectrophotometer on a regular basis to ensure the reliability of the results. A general understanding of the effects of each performance attributes on the outcome of the measurements will help to improve UV-Vis experimentation. So NIS-Egypt maintains the national scales for absorbance and transmittance in ultraviolet and visible spectral regions.

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紫外可见光谱区分光光度计的标准化

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摘　要： 紫外可见光谱测量通常用于实验室的定性和定量分析。为确保测量值可靠，实验仪器必须经过校准。埃及国家标准化所建立了一种光度测定法，其吸收和传输能力满足国际和国家要求。本文介绍了紫外可见光谱测及测量仪，讨论了影响测量可靠性的因素，并讨论了包含相关测量参数的不确定度模型。

关键词： 分光光度计；标准化；吸收；不确定度

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