## SPECIAL ISSUE PAPER

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# The use of spectro(photo)metric reference materials in chemical metrology – present status in Romania

**Abstract** This paper discusses some aspects of the use of certified reference materials (CRMs) to ensure the uniformity of results, especially through calibration and validation of spectro(photo)metric instrument performance. In this way the link between the intrinsic performance of instruments and the accuracy of measurement results is underlined. Some types of local CRM are also presented.

## Introduction

There is a great need to ensure the uniformity and comparability of measurements in every day activities. Many important decisions are based on results from quantitative analysis and results of high quality are required. The quality of results depends on a variety of factors, among which the measurement methods, the measuring instruments, and the appropriate CRMs are some of the most important. Reference materials are used and their use as measurement standards is widely stipulated in documented methods in many fields of activity. For this reason our laboratory has developed spectro(photo)metric reference materials for use in metrological assurance of spectrometric results in accordance with the Romanian law of metrology.

## Local reference materials used in UV–Vis spectrophotometry

In accordance with international recommendations [1, 2] CRMs are used to calibrate and verify measuring equipment, to evaluate methods of measurement, to determine by comparative methods the quantities characterizing the composition or properties of substances and materials, and to evaluate the uncertainty of measurement results, etc.

The mission of our metrological laboratory is to establish the scientific background for uniformity, consistency, and accuracy of chemical measurements in Romania. Spectrometric methods are extensively used in our laboratories. Because ultraviolet–visible molecular spectro(photo)metry (UV–Vis), atomic absorption spectrometry (AAS), and inductively coupled plasma–atomic emission spectrometry (ICP–AES) are commonly employed and versatile analytical techniques, it is important to define technical specifications that enable selection of the correct instrument for individual applications and maintenance of optimum spectrometer performance in daily routine analysis.

To ensure the national uniformity and accuracy of these kinds of measurements, our institute has developed certified reference materials for optical properties (molecular absorption, wavelength, photometric linearity, and stray radiant energy), which play a major part in chemical measurements performed in Romania. Some aspects of the destination, description, assigned values, and associated uncertainties of these kinds of CRMs are presented in Table 1. The spectrophotometric solutions were calibrated on a UV–Vis spectrophotometer used only for calibration purposes and quality control checking of this instrument was performed in accordance with Ref. [3] using a set of Unicam No. 9423 179 38091 standards. Certification of reference materials by the metrological approach enables these to be used reliably in the statement of uniformity and comparability of analytical measurements.

## Verification of the performance of UV–Vis spectrophotometers

The quality of analytical results directly depends on the accuracy of analytical methods and on a variety of other factors, the most important of which is the instrumentation. As far as spectrophotometric performance in UV–Vis

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**Table 1** Some types of spectrophotometric reference material

| Code CRMs | Description of CRMs | Destination of CRMs | Certified values of CRMs (P: 95%) | Traceability of certified values against |
|---|---|---|---|---|
| 10,01 | 5-mL vial (holmium oxide solution) | Verification and/or calibration of the wavelength scale of UV–Vis spectrophotometers, uncertainties within the range (0.3...2.0) nm | $\lambda$: (240...540) nm $u_\lambda$: (0.3...0.7) nm | Perkin–Elmer standard no. 1601 |
| 10,02 | 5-mL vial (cobalt chloride solution) | | | |
| 10,03 | Filter type BG35 | | | |
| 10,04 | 5-mL vial benzene | | | |
| 11,01 | Two 5-mL vials (potassium dichromate) | Verification and/or calibration of the absorbance scale of UV–Vis spectrophotometers, uncertainties within the range (0.005...0.009) nm between 235 nm and 721 nm | $u_\lambda$: 0...2.000 $u_A$: (0.005...0.009) | INM standard no. 172 |
| 11,02 | Three 5-mL vials (nickel nitrate) | | | |
| 11,03 | Three 5-mL vials (cobalt nitrate) | | | |
| 11,04 | Three 5-mL vials (nickel nitrate + cobalt chloride ) | | | |
| 15,01 | 10-mL bottle NaI | Stray light evaluation within the spectral range (220...650) nm | $\lambda$: 220 nm, T < $10^{-4}$% $\lambda$: 370 nm, T < $10^{-4}$% $\lambda$: 650 nm, T < $10^{-4}$% | Perkin–Elmer standards no. B050–5100, no. B050–1541, no. B050–5070 |
| 15,02 | 10-mL bottle NaNO$_2$ | | | |
| 15,03 | 10-mL bottle methylene blue | | | |
| 16,01 | Five 5-mL vials (potassium dichromate) | Photometric linearity check of the UV–Vis spectrophotometers, uncertainty of 1% | $\lambda$: (235...512) nm A: 0...1.500 $u_A$: (0.005...0.009) | INM standard no. 172 |
| 16,05 | Five 5-mL vials (cobalt-ammonium sulphate) | | | |

spectrophotometry is concerned, it is an essential criterion that the spectral data are accurate and reproducible. The national, legal and technical metrological norms issued by our institute cover UV–Vis spectrophotometers [3]. The main instrumental performance of UV–Vis spectrophotometers and some of their effects of the specification on the analytical results are presented.

1. Wavelength accuracy. The term "wavelength range" describes the wavelength-producing ability of a spectrophotometer in terms of upper and lower limit. All wavelengths within this range must be of sufficient energy for proper performance. A UV–Vis spectrophotometer typically covers the wavelength range between 190–850 nm.

Most commonly used in environmental laboratories are visible-region spectrophotometric devices that cover the range 350–800 nm. The absorbance of most samples is sufficiently wavelength-dependent that even in the most favorable regions of maximum and minimum absorbance a wavelength error of 1–2 nm can produce absorbance errors of a few tenths of a percent.

2. Photometric accuracy is a measure of the absolute correctness of an absorbance or transmittance value, depending on the spectral bandwidth. Also, photometric accuracy is highly correlated with photometric repeatability, which specifies the deviation of an absorbance value in repeated measurements.

**Table 2** Validation of instrument performances for UV–Vis spectrophotometers

| Instrumental performance | Instrument types[a] | Code CRMs | Certified values of CRMs | Measured value | Calculated errors | Permitted errors[b] |
|---|---|---|---|---|---|---|
| Wavelength accuracy, nm | 1 | 10,01 | 241.1 ± 0.3 | 241.8 | + 0.7 | ± 1.0 |
| | 2 | 10,01 | 451.2 ± 0.3 | 451.6 | + 0.4 | ± 0.5 |
| | 3 | 10,01 | 640.6 ± 0.5 | 640.2 | − 0.4 | ± 0.5 |
| Photometric accuracy | 1 | 11,04 | 0.452 ± 0.002 | 0.455 | + 0.003 | ± 0.007 |
| | 2 | 11,01 | 0.862 ± 0.010 | 0.868 | + 0.006 | ± 0.007 |
| | 3 | 11,02 | 0.215 ± 0.004 | 0.217 | + 0.002 | ± 0.007 |
| Stray light, % | 1 | 15,02 | < $10^{-4}$% (T) | 3.2 | 0.06 | < 0.10 |
| | 2 | 15,01 | < $10^{-4}$% (T) | 3.1 | 0.08 | < 0.10 |
| | 3 | 15,01 | < $10^{-4}$% (T) | 3.4 | 0.04 | < 0.05 |
| Linearity, % | 1 | 16,03 | 0.613 ± 0.005 | 0.005 | 0.8 | < 1.0 |
| | 2 | 16,02 | 0.305 ± 0.005 | 0.308 | 1.0 | < 1.0 |
| | 3 | 16,01 | 0.155 ± 0.005 | 0.156 | 0.6 | < 1.0 |

[a] Instrument types: 1, DR 2000 single-beam spectrophotometer; 2, Specord M40 double-beam spectrophotometer; 3, Hewlett–Packard 8452A diode-array spectrophotometer
[b] Permitted errors are calculated by use of the equations:
$e_\lambda = \lambda_m - \lambda_{CRM}$ where $\lambda_m$ is measured wavelength and $\lambda_{CRM}$ is certified value of wavelength

$e_A = A_m - A_{CRM}$ where $A_m$ is measured absorbance, $A_{CRM}$ is certified value of absorbance
$e_T = 10^{-A} * 100$ where $A = A_{(attenuator\ filter)} + A_{CRM\ (cut-off\ filter)}$ where $A$ is the measured absorbance
$e_L = (A_m - A_{CRM}) * 100 / A_{CRM}$ where $A_m$ is measured absorbance, $A_{CRM}$ is certified value of absorbance

3. Stray radiant energy is any unwanted radiant energy measured by the detector that is outside the spectral region isolated by the bandwidth of the instrument. A large amount of stray radiation can severely limit linearity. When stray radiation is present and not absorbed by the sample, the absorbance reading might be low. The effect has only a small influence at low absorbance values, but with increasing absorbance the stray radiation become more and more dominant and the absorbance reading becomes too low. Consequently the stray radiation specifications have a direct influence on the calibration curves and thus on the precision of the results.

4. Photometric linearity is a necessary, but not sufficient, condition to establish the linear relationship between absorbance and concentration. Thus a general check of the instrument performance is to confirm that a solution known to conform to the Beer–Lambert Law gives a linear plot of absorbance against concentration.
Validation of instrumental performance for some types of UV–Vis spectrophotometer is presented in Table 2. In this way, knowing the performance of instruments, and taking into account the Lambert–Beer Law, it is possible to choose an appropriate instrument for a specific analytical purpose [3].

## The spectrometric reference materials used in chemical metrology

The most common methods used in national chemical metrology are AAS and ICP–AES. The spectrometric reference materials or single-element standard solutions are used to evaluate instrumental performance and to calibrate the concentration scale of the spectrometer. These standard solutions are prepared gravimetrically [4] from high-purity metals and salts by measuring the masses or volumes of the different pure materials. They are certified with regard to the accuracy [5] of their major constituents using titrimetry as a measurement method that ensures traceability to primary standards or at least to well-characterized and recognized secondary standards.

Some aspects of the certification
of spectrometric reference materials

Concentration measurements of spectrometric reference materials are performed by compleximetric titration and by spectrometric reference methods (e.g. AAS or ICP–AES).

The compleximetric method involves the titration of a sample solution against a Complexon III (EDTA) solution ($0.05$ mol $L^{-1}$) standardized with calcium carbonate ($CaCO_3$; SRM 915 a).

The concentration, c, is calculated from:

$$c = \frac{1000 \cdot m_{CaCO_3} \cdot y_{CaCO_3}}{V_f \cdot M_{CaCO_3}} \frac{V_{CaCO_3}}{V_{1,Cx}} \frac{V_{2,Cx}}{V_{sp}}$$

where c is the concentration of the sample (mol $L^{-1}$), $m_{CaCO_3}$ is the weight of $CaCO_3$ (g), $y_{CaCO_3}$ is the purity of

$CaCO_3$ (mass fraction), $V_f$ is the volume of the $CaCO_3$ solution ($cm^3$), $M_{CaCO_3}$ is the molar-mass of $CaCO_3$ (g $mol^{-1}$), $V_{CaCO_3}$ is the volume of the $CaCO_3$ solution ($cm^3$), $V_{1,Cx}$ is the volume of the titrated Complexon III solution ($cm^3$), $V_{2,Cx}$ is the volume of the Complexon III solution delivered from the burette ($cm^3$), and $V_{sp}$ is the volume of the titrated sample solution ($cm^3$).

The titrimetric measurement involves a series of steps in which the calculated output value from one step is an input value for a succeeding step. To estimate the uncertainty of a titrimetric measurement [6] it is necessary to identify the steps of measurement process. Figure 1 shows the percentage of the components corresponding to the variances as proportions of the overall variance for the calcium concentration measurement [7]: the percentage of the variance in the overall variance of end-point detection, $e_{t,1}$ and $e_{t,2}$ are significant (3.53%) but not essential; the percentage of the variance of volume measurement $V_{1,Cx}$, $V_{sp}$ (both 36.00%) and $V_{2,Cx}$ (14.68%) are predominant and must be reduced by using class A volumetric glassware; the percentage of the variance of the calcium carbonate solution $V_{CaCO_3}$ delivered from burette is 3.68%; the percentage of the variance of the primary reference materials purity $y_{CaCO_3}$, is 2.10% and, finally, the percentage of the variance of weighing the initial substance $m_{CaCO_3}$, of the volume of the final calcium carbonate solution, $V_f$, of calcium carbonate molar mass $M_{CaCO_3}$, are insignificant and can be neglected.

The extent to which each of these components contributes to the overall variability is obviously important when trying to reduce the process variability and improve the analytical process.

The spectrometric solutions for Cu, Cd, Co, Fe, Mg, Pb, Zn etc. characterized in this way are used to evaluate the analytical performances of AAS instruments. The main analytical performance characteristics of AAS instruments are:

1. Sensitivity (characteristic concentration): The term "sensitivity" has a special meaning in AAS and is defined as that concentration of the element in aqueous solution, which gives an absorbance of 0.0044, equal to a 1% absorption of the transmitted radiation. Knowledge of the sensitivity value for a particular element is useful, be-
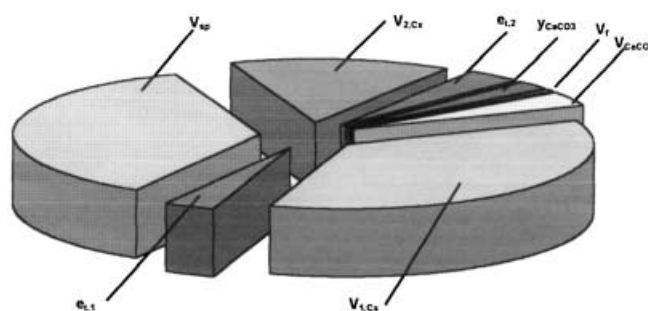


**Fig. 1** Variance components of calcium concentration measurement by titrimetry

**Table 3** Evaluation of the analytical performance of some AAS instruments

| Analytical performance | Varian AA 250 Plus | Unicam Sollar 939 | AAS 30 C.Z. Jena | AAS 3 C.Z. Jena |
|---|---|---|---|---|
| Calibration parameters: | | | | |
| Intercept, a | 0.0160 | 0.0160 | 0.0116 | 0.0255 |
| Standard deviation of the intercept, $s_a$ | 0.0034 | 0.011 | 0.0025 | 0.0119 |
| Slope, b | 0.0634 | 0.0846 | 0.0605 | 0.0665 |
| Standard deviation of the slope, $s_b$ | 0.0007 | 0.002 | 0.0004 | 0.0018 |
| Correlation coefficient, r | 0.9991 | 0.9989 | 0.9998 | 0.9988 |
| Standard deviation of the regression, $s_0$ | 0.0091 | 0.0150 | 0.0035 | 0.0116 |
| Characteristic concentration, mg $L^{-1}$ | 0.060 | 0.048 | 0.066 | 0.074 |
| Detection limit, mg $L^{-1}$ | 0.0025 | 0.0028 | 0.0032 | 0.0036 |

cause from this the optimum concentration range for that element may be computed.

2. Detection limit is a statistical function which takes into account blank levels, sensitivity, and signal-to-noise ratios. The detection limit is defined as that concentration of an element which gives a reading equal to three times the standard deviation of a signal measured to the blank level. As a consequence, some linear calibration curve, sensitivity, and detection limit results for determination of copper by different types of flame atomic absorption spectrometers are presented in Table 3. The standard deviation of the linear regression dispersed within a large range (0.0035...0.0150) indicates that more than five standard solutions and three replicates must be used for linear calibration in the range of interest. The characteristic concentration values for copper determination are dispersed within a range (0.048...0.074) mg $L^{-1}$ and this provides useful information about the intrinsic performance of the spectrometers and enables the operator to predict the absorbance range which will be observed for a specific sample concentration range. The detection limits for copper determination are contained within the range (0.0025...0.0036) mg $L^{-1}$; this depends on the properties of the calibration curve.

## Conclusions

In Romanian chemical metrology spectro(photo)metric CRMs play an important role in ensuring the uniformity of measurement results. The main types of CRMs with optical properties used for metrological verification of UV–Vis spectrophotometers are pointed out, emphasizing the possibility of ensuring their traceability. Their use in the validation of instrumental performance and the link between intrinsic performance and the accuracy of results are also presented.

The representative spectrometric standard solutions used in AAS method are described, with emphasis on ways of metrological characterization by primary methods. Some results from the testing of instrumental performance, especially the sensitivity and the detection limit of different types of AAS instrument are also presented, with emphasis on factors that influence their evaluated values.

## References

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