National Institute of Standards & Technology

Certificate

Standard Reference Material 931e

Liquid Absorbance Standard for Ultraviolet and Visible Spectrophotometry

This Standard Reference Material (SRM) is intended primarily for routine critical evaluation of daily working standards used in spectrophotometry, and for use in the calibration and checking of accuracy of the photometric scale of spectrophotometers that provide a narrow effective spectral bandpass, not to exceed 1.5 nm at 302 nm, 2.0 nm at 395 nm, 3.3 nm at 512 nm, and 8.5 nm at 678 nm. [1]

SRM 931e is certified as solutions of known net absorbances at four (4) specific spectral wavelengths for a 10.00-mm measurement pathlength (see Preparation of Filter Solutions for details). Each unit of SRM 931e consists of 3 sets of liquid filters (12 ampoules total), each set consisting of a blank solution and 3 absorbance levels (I, II, and III) of the filter (nominal absorbances of 0.3, 0.6, and 0.9, respectively, for a 10-mm pathlength). Approximately 10 mL of each liquid filter is individually flame-sealed in a glass ampoule which has been prescored for easy opening. Each set of liquid filters in the SRM unit is individually packaged in a tray.

The certified net absorbances are given below for the three solution levels at four wavelengths, for a 10.00-mm pathlength cell and a temperature of 22 ± 1 °C. The uncertainties of the certified values include all known sources of random and possible systematic errors (see Certification of Net Absorbance for 10.00-mm pathlength).

	Wavelength, nm			
Filter	302	395	512	678
Level I	0.2978 ± 0.0015	0.3060 ± 0.0015	0.3013 ± 0.0015	0.1153 ± 0.0015
Level II	0.5946 ± 0.0025	0.5954 ± 0.0025	0.6001 ± 0.0025	0.2245 ± 0.0015
Level III	0.9160 ± 0.0035	0.9009 ± 0.0035	0.9055 ± 0.0035	0.3402 ± 0.0015

Note: All certified values have been corrected for absorbances due to the blank solution.

The preparation of the filter solutions and the performance of the transmittance measurements for the certification process were performed in the NIST Inorganic Analytical Research Division by J.D. Messman.

The overall coordination of technical measurements leading to certification was performed in the NIST Inorganic Analytical Research Division by R.L. Watters, Jr. and J.C. Travis.

The statistical analysis of the data was performed by S.B. Schiller of the NIST Statistical Engineering Division.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by J.C. Colbert.

Gaithersburg, MD 20899
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(Revision of certificate dated 10-20-86)

William P. Reed, Chief
Standard Reference Materials Program

(over)

NOTICE AND WARNINGS TO USER

Stability and Expiration of Certification: While no long-term stability studies have been made on this lot (931e), studies on previous lots (931, 931a, 931b, and 931c) over three-year periods showed no degradation of the material when stored in the original sealed ampoules. Therefore, this material is certified only for use within three years following the date of shipment from NIST.

Preparation of Filter Solutions: The filter solutions were prepared by dissolving high-purity cobalt and nickel in a mixture of nitric and perchloric acids. The absorbance of nitrate ion was adjusted to a comparable level by evaporation and, if necessary, subsequent addition of small amounts of nitric acid. The nominal weights of the cobalt and nickel metals and the typical volumes of nitric and perchloric acids used in the preparation of the three absorbance levels of solutions are given below for information.

Liquid Filter	Cobalt (g)	Nickel (g)	HNO_3 (mL)	$HClO_4$ (mL)
Level I	7.4	6.9	30	50
Level II	14.7	13.9	45	90
Level III	22.1	20.7	65	125

To facilitate dissolution of the metals and removal of excess nitric acid, the requisite weights of metal needed to produce the 20-L volume of solution for each absorbance level were divided into ten equal portions.

The dissolutions were performed in 1-L glass beakers. Following dissolution and evaporation of excess nitric acid for each portion on a hot plate, the solutions were filtered through Whatman No. 2 filters directly into 2-L volumetric flasks to remove any residual insoluble particles. The solutions were then diluted to calibrated volume with distilled water and mixed thoroughly. The ten 2-L portions for each absorbance level were combined in a 20-L glass carboy and the contents were swirled thoroughly to produce the final solutions. The blank solution (0.1N $HClO_4$ in distilled water) was produced by preparing ten 2-L portions of distilled water containing 18 mL of $HClO_4$ and combining the portions in a 20-L glass carboy.

Following preparation of the 20-L volumes of the blank solution (0.1N $HClO_4$) and the three absorbance level solutions, the solutions were ampouled, each containing approximately 10 mL of solution.

The maxima in the absorbance spectrum (see figure 1) at 302 and 512 nm are due to absorbance by NO_3^- and $Co(H_2O)_6^{++}$, respectively. The maximum at 395 nm and the plateau at 650-700 nm are due to $Ni(H_2O)_6^{++}$. The pH of these solutions is about 1.

Certification of Net Absorbance for 10.00-mm Pathlength: The transmittance measurements leading to the certification of this SRM were performed at an ambient temperature of 22 ± 1 °C using the NIST high accuracy spectrophotometer. The design and construction of this instrument have been described previously.[2] The instrument is a primary transmittance standard; its accuracy has been verified using the double aperture radiation-addition principle. The effective spectral bandpass used to determine the certified values was 0.8 nm. The transmittance measurements were made by producing the vertical image of the slit (about 8 mm by 0.5 mm), using a convergent beam geometry with an aperture ratio f:10, in the middle of the entrance face of the sample cuvette. The cuvette was oriented in a position perpendicular to the incident light beam.

The liquid absorbance filter solutions were calibrated at the wavelengths and conditions indicated by measuring the transmittance, T, of the blank and solutions I, II, and III against air as a reference. Each transmittance measurement is calculated from a measurement of the intensity transmitted through the filter and bracketing measurements of the intensity transmitted through an empty filter holder, with a settling time of approximately 5 s and a signal integrating time of approximately 1 s for each measurement.

For the certification measurements, 18 ampoules were randomly selected from each of the four solutions (blank and 3 absorbance levels). Randomized measurements were made in four runs on each of three days. In each run, six unknown samples (SRM 931e) and one control sample (SRM 931d) were measured in triplicate using cuvettes with the pathlength known to ± 0.0005 mm. On each day, six samples from each of the four levels (blank and levels

I, II, and III) were measured. Two samples from three of the four levels were measured in each run. A blank was run in each cuvette on each day.

The values of T were used to calculate the corresponding values of the apparent absorbance, A, using the relationship $A = -\log_{10}T$. The net absorbances were obtained by subtracting the apparent absorbance of the blank solution from the apparent absorbances calculated for solutions I, II, and III. The certified net absorbances are the grand mean values for the three levels and four wavelengths. Statistical analysis revealed no apparent "day" or "cuvette" effects in the data. A small apparent "run" effect was consistent with the temperature coefficients given below, and with the normal temperature variation in the laboratory. The random error (95% confidence interval) was about a third of the total estimated error given in the table, which includes systematic error estimates to accommodate a $\pm$ 1 °C temperature variation and a small angular uncertainty in cuvette orientation.

Temperature Dependence: Absorbances at various temperatures (17° to 35 °C) may be calculated using the equation:

$$A_T = A_{22}[1+C_A(T-22)]$$

where: A_T = Absorbance at temperature T (°C)
A_{22} = Absorbance certified at 22.0 °C
C_A = Fractional change in absorbance per °C

The values of C_A, at the four wavelengths, are given below.

Note: At wavelength 302 nm, absorbance decreases with increasing temperature; at the other wavelengths, absorbance increases with increasing temperature.

Wavelength, nm	C_A
302	-0.0014
395	+0.0014
512	+0.0018
678	+0.0014

Wavelength Accuracy and Bandpass Requirements: Due to the spectral features in the absorbance spectrum (see figure 1), the absorbances of these liquid absorbance standards will depend not only on the accuracy of the photometric scale, but also on the wavelength accuracy and the spectral bandpass of the spectrophotometer. A mercury lamp is recommended for checking the wavelength scale. In addition, for those spectrophotometers having a hydrogen (H) or deuterium (D) source, the two emission lines at 486.1 and 656.3 nm (H) or 486.0 and 656.1 nm (D) may provide a convenient check at these wavelengths. To ensure that the measured absorbances are not significantly different from the certified values, the wavelength scale of the spectrophotometer should be calibrated to within $\pm$ 0.5 nm, and the effective spectral bandpass should not exceed 1.5, 2.0, 3.3, and 8.5 nm at 302, 395, 512, and 678 nm, respectively.

Instructions for Use: This Standard Reference Material should be kept in the original sealed ampoules. Once opened, the material should be used immediately. No attempt should be made to reseal the ampoule.

The instructions below are for use with the standard 10-mm rectangular cuvette. For calibration of the several spectrophotometric systems used in various automated instruments, the user is referred to the instruction manual for the particular instrument.

1. Select two clean 10.00-mm cuvettes free of scratches. At least one should be fitted with a ground glass or inert stopper to minimize evaporation. Reserve it for all sample measurements.

2. Mark each of the cuvettes to assure the same orientation in the spectrophotometer.

3. Place the cuvettes in their respective holders and fill with distilled water. (Borosilicate Pasteur-type pipettes fitted with rubber bulbs are recommended for transferring all solutions to and from the cuvettes. Soft glass pipettes, which are available commercially, contain residual amounts of ultraviolet absorbing material, but may be used after proper cleaning. Several rinses, first with isopropyl alcohol and then with distilled water, are generally adequate).

4. Obtain the optical mismatch of the cuvettes at 302, 395, 512, and 678 nm, using the effective spectral bandpass limitations not to exceed 1.5 nm at 302 nm, 2.0 nm at 395 nm, 3.3 nm at 512 nm, and 8.5 nm at 678 nm. [1]

5. Empty the cuvettes by suction without removing them from their holders, refill with distilled water and measure the absorbances again at each of the above wavelengths.

6. Repeat the emptying and refilling operation until constant absorbance readings are obtained.

7. Using the liquid filters provided, measure, in turn, the absorbance of the blank and solutions I, II, and III against distilled water. Shake each ampoule before opening to remix any condensate which may have been collected in the neck (the ampoules have been prescored directly below the gold band to facilitate opening).

8. Subtract the appropriate blank reading from the absorbances obtained for I, II, and III. These net absorbances should agree with the certified values within the uncertainties specified. Consult the manufacturer of the instrument if they do not.

REFERENCES

[1] Burke, R.W., Deardorff, E.R., and Menis, O., J. Research, Nat. Bur. Stand. 76A, 469-482 (1972).

[2] Mavrodineanu, R., J. Research, Nat. Bur. Stand. 76A, 405-425 (1972).

Note: References [1, 2] are also published in NBS Special Publication 378, Accuracy in Spectrophotometry and Luminescence Measurements, R. Mavrodineanu, J.I. Shultz, and O. Menis, Editors, U.S. (1973).

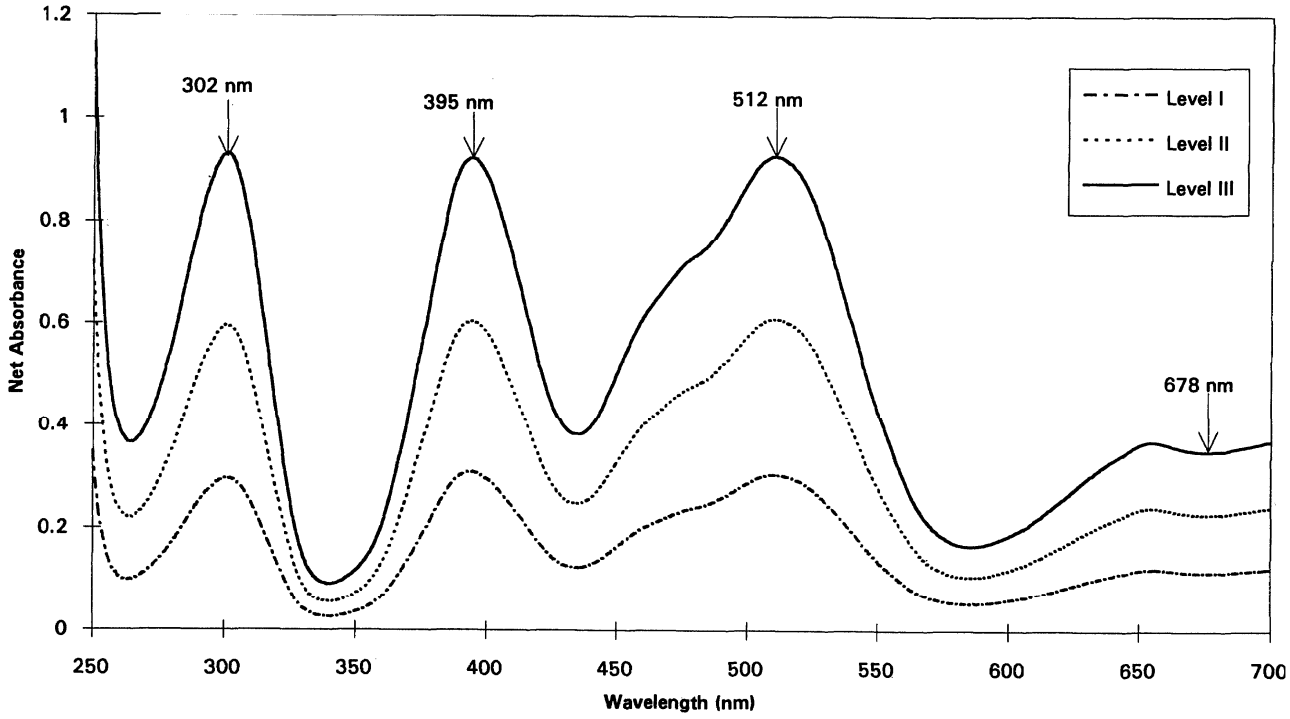

Figure 1. Absorbance Spectrum of SRM 931e