



# Certificate of Analysis

## Standard Reference Material<sup>®</sup> 931h

### Liquid Absorbance Standard for Ultraviolet and Visible Spectrophotometry

This Standard Reference Material (SRM) is intended primarily for critical evaluation of daily working standards used in spectrophotometry and for use as an accuracy check of the photometric scale of spectrophotometers that provide a narrow effective spectral bandpass not to exceed the following: 2.2 nm at 302 nm, 2.9 nm at 395 nm, 4.8 nm at 512 nm, and 12.3 nm at 678 nm [1]. A unit of SRM 931h consists of three kits, each comprising three cobalt-nickel liquid filter solutions at different concentrations (Level I, Level II, and Level III) and a blank solution (12 ampoules total). Each sealed borosilicate glass ampoule contains approximately 10 mL of solution.

**Certified Absorbances:** The measurand is the net absorbance at four specific wavelengths for a 10.00 mm measurement pathlength (see “SRM Filter Preparation”). Metrological traceability is to the SI base unit of length and to the decadic logarithm of the derived SI unit of regular spectral transmittance, expressed as absorbance. The three liquid filters, referred to as Levels I, II, and III, have nominal absorbances of 0.3, 0.6, and 0.9 at three certification wavelengths. The certified net absorbances, corrected for the absorbance due to the blank solution, are given in Table 1 for the three liquid filters at four wavelengths for a 10.00 mm pathlength cell and a temperature of  $22\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ . The uncertainties of the certified values include all known sources of random and possible systematic errors (see “Certification of Net Absorbance”). The uncertainties listed in Table 1 are expanded uncertainties, calculated according to the ISO/JCGM Guide [2] at the 95 % level of confidence. These uncertainties include contributions due to: random variation in the spectrophotometer measurements; a sample temperature variation of  $\pm 1\text{ }^{\circ}\text{C}$ ; finite bandwidth effects; and baseline bias.

Table 1. Certified Absorbances and Uncertainties for SRM 931h<sup>(a)</sup>

Liquid Filter	Nominal Wavelength (nm)			
	302	395	512	678
Level I	$0.2945 \pm 0.0016$	$0.3162 \pm 0.0017$	$0.3024 \pm 0.0017$	$0.1181 \pm 0.0012$
Level II	$0.5487 \pm 0.0026$	$0.6390 \pm 0.0030$	$0.5984 \pm 0.0029$	$0.2405 \pm 0.0019$
Level III	$1.0924 \pm 0.0049$	$0.9667 \pm 0.0044$	$0.9224 \pm 0.0042$	$0.3761 \pm 0.0026$

<sup>(a)</sup> All certified values have been corrected for the absorbance due to the blank solution.

**Expiration of Certification:** The certification of **SRM 931h** is valid, within the measurement uncertainty specified, until **31 December 2032**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Handling, Storage, and Use”). The certification is nullified if the SRM is damaged, contaminated, or modified.

**Maintenance of SRM Certification:** NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall coordination of technical measurements leading to the certification of SRM 931h was provided by S.J. Choquette of the NIST Office of Reference Materials.

Filter solution preparation and original transmittance measurements were performed by M.V. Smith and J.C. Travis (Emeritus) of the NIST Biosystems and Biomaterials Division.

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Stability data analysis and temperature coefficient consultation were performed by D.L. Duewer of the NIST Chemical Sciences Division.

Statistical analysis of the experimental data was performed by H.-k. Liu of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

## PREPARATION AND ANALYSIS

**SRM Filter Preparation:** The filters were prepared by dissolving high purity cobalt and nickel in a mixture of reagent grade nitric and perchloric acids and diluting in distilled water. The blank was prepared as 9 mL/L perchloric acid in distilled water. The absorbance spectrum of the resulting solutions is shown in Figure 1. The maxima at 302 nm and 512 nm are due to absorbance by  $\text{NO}_3^-$  and  $\text{Co}(\text{H}_2\text{O})_6^{+2}$  respectively. The maximum at 395 nm and the plateau in the 650 nm to 700 nm range are due to  $\text{Ni}(\text{H}_2\text{O})_6^{+2}$ . The pH of these solutions is approximately 1.

**Certification of Net Absorbance:** The transmittance measurements leading to the certification of this SRM were performed at a sample temperature of  $23.5\text{ }^\circ\text{C} \pm 0.5\text{ }^\circ\text{C}$  using the second-generation NIST high accuracy spectrophotometer. The design and construction of this instrument follows that of the original [3]. The instrument is a primary transmittance standard; its accuracy has been verified using the double aperture radiation addition principle. The effective spectral bandwidth used to determine the certified values was 0.8 nm. The transmittance measurements were made by producing the vertical image of the slit (approximately  $4\text{ mm} \times 1\text{ mm}$ ), using convergent beam geometry with an aperture ratio  $f/11$ , 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 filter solutions were calibrated at the wavelengths and conditions indicated by measuring the transmittance,  $T$ , of the blank and Levels I, II, and III solutions referenced to air. Each transmittance measurement is calculated from a measurement of the intensity transmitted through a 10 mm path length cuvette containing the SRM solution and bracketing measurements of the intensity transmitted through an empty cuvette 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 using path lengths corrected to  $10.000\text{ mm} \pm 0.001\text{ mm}$ , 18 ampoules were randomly selected from each of the four solutions (blank and three absorbance levels). Randomized measurements were made in four runs on each of three days. In each run, six unknown samples (SRM 931h) and one control sample (SRM 931g Liquid Absorbance Standard for Ultraviolet and Visible Spectrophotometry) were measured in triplicate. 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 Levels I, II, and III and corrected to a 10.000 mm pathlength and a temperature of  $22.0\text{ }^\circ\text{C}$ . The certified net absorbances are the grand mean values for the three levels and four wavelengths.

**Temperature Dependence:** Temperature affects the absorbance of these standards. Temperatures above  $25\text{ }^\circ\text{C}$  should be avoided. Absorbances at various sample temperatures ( $17\text{ }^\circ\text{C}$  to  $25\text{ }^\circ\text{C}$ ) may be corrected to the certification temperature using the equation:

$$A_{22} = A_t/[1+C_A(t-22)]$$

where:  $A_{22}$  = Absorbance corrected to  $22.0\text{ }^\circ\text{C}$   
 $A_t$  = Measured absorbance at temperature  $t$  in  $^\circ\text{C}$   
 $C_A$  = Fractional change in absorbance per  $^\circ\text{C}$   
 $t$  = Sample temperature

The values of  $C_A$  at the four certification wavelengths are given in Table 2.

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

Table 2. Temperature Coefficients at Certification Wavelengths

Nominal Wavelength, (nm)	C <sub>A</sub>
302	– 0.0013 ± 0.0002
395	+ 0.0013 ± 0.0001
512	+ 0.0015 ± 0.0001
678	+ 0.0013 ± 0.0002

**Wavelength Accuracy and Bandpass Requirements:** Due to the spectral features in the absorbance spectrum (see Figure 1), the absorbances of these liquid filters will depend not only on the accuracy of the photometric scale, but also on the wavelength accuracy and the spectral bandwidth of the spectrophotometer. A holmium oxide solution wavelength standard such as NIST SRM 2034 Holmium Oxide Solution Wavelength from 240 nm to 650 nm is recommended for calibrating the wavelength scale. In addition, for those spectrophotometers having a hydrogen (H<sub>2</sub>) or deuterium (D<sub>2</sub>) source, the two emission lines at 486.1 nm and 656.3 nm (H<sub>2</sub>) or 486.0 nm and 656.1 nm (D<sub>2</sub>) 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 ± 0.5 nm, and the effective spectral bandwidth should not exceed 2.2 nm, 2.9 nm, 4.8 nm, and 12.3 nm at 302 nm, 395 nm, 512 nm, and 678 nm, respectively.

#### INSTRUCTIONS FOR HANDLING, STORAGE, AND USE<sup>(1)</sup>

**WARNING:** This SRM is an acid solution contained in tip-sealed borosilicate glass ampoules with pre-scored stems. Therefore, all appropriate safety precautions, including use of gloves during handling, should be taken to avoid injury or spillage.

**Opening an Ampoule:** When an ampoule is to be opened, that area of the stem where the pre-scored band is located should be carefully wiped with a clean, damp cloth and the body of the ampoule wrapped in absorbent material. The use of gloves for simultaneous protection from chemical contact and sharp glass edges is recommended. Holding the ampoule steady and with thumb and forefinger grasping the stem, **minimal** thumb pressure should be applied to the stem to snap it. Correctly done, the stem should break easily where pre-scored. Use of a metal file to break the stem is **NOT** recommended.

**Storage:** SRM 931h should be kept in the original sealed ampoules until use. Unopened ampoules should be stored under normal laboratory conditions in an upright position inside the original container supplied by NIST. Once opened, the solutions should be used immediately. No attempt should be made to reseat the ampoules.

**Use:** The instructions below are for use with the standard 10 mm pathlength rectangular cuvette and apply to either single beam or double beam spectrophotometers. For validation of spectrophotometric systems used in various automated instruments, the user should refer to the instruction manual for the particular instrument.

- Step 1. Reserve at least one 10 mm cuvette of known pathlength uncertainty for all SRM measurements. Cuvettes should be clean, free of scratches, and fitted with ground-glass or Teflon<sup>®</sup> stoppers. A single cuvette (into which a liquid filter can be pipetted) may be used and left in place in the sample chamber of the spectrophotometer to minimize sources of variability due to cuvette pathlength, positioning, etc. Alternatively, the use of four stoppered matched cuvettes permits randomized replication over the course of a day.
- Step 2. Mark each cuvette to assure reproducible orientation in the spectrophotometer in case the cuvette is removed and replaced during the calibration procedure.
- Step 3. Rinse each cuvette repeatedly with deionized organic-free water, making apparent absorbance readings (referenced to air) at 302 nm after each rinse, until constant absorbance readings are obtained. Borosilicate Pasteur-type pipettes fitted with rubber bulbs are recommended for transferring solutions to

<sup>(1)</sup>Certain commercial instruments, materials, or processes are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the instruments, materials, or processes identified are necessarily the best available for the purpose.

and from the cuvettes. (Commercially available soft glass pipettes may contain residual amounts of ultraviolet absorbing material, but may be used after proper cleaning. Several rinses, first with isopropyl alcohol and then with deionized organic-free water, are generally adequate.)

- Step 4. Using the liquid filter solutions provided, measure the apparent absorbance of the blank and of Levels I, II, and III against air in the following manner. Assure that the solutions and cuvettes are temperature-equilibrated to the laboratory or sample chamber before measurement. If the sample chamber differs by more than  $\pm 1^\circ\text{C}$  from room temperature during the calibration, either the data should be collected quickly or the sample be allowed to equilibrate to the sample chamber temperature. Record the appropriate temperature. Shake each ampoule before opening to remix any condensate that may have been collected in the neck. Rinse the sample cuvette with at least two complete fillings of the SRM solution from each 10 mL ampoule before taking final apparent absorbance readings for that ampoule at nominal wavelengths of 302 nm, 395 nm, 512 nm, and 678 nm, using an effective spectral bandpass not to exceed 2.2 nm, 2.9 nm, 4.8 nm, and 12.3 nm, respectively. (Unless wavelength accuracy to within 0.5 nm can be verified with SRM 2034, absorbance maxima in the vicinity of the shortest three wavelengths should be used to test the absorbance accuracy of the instrument independently of the wavelength accuracy.) Solutions from the rinses should be collected for proper disposal. If four cuvettes are used, replication is recommended with removal and replacement of cuvettes between runs and a randomized run order for the four solutions. **Validation must be performed the same day when using solutions prepared in stoppered cuvettes due to the possibility of evaporative losses.**
- Step 5. For each wavelength and replication subtract the apparent absorbance of the blank from the apparent absorbances obtained for Levels I, II, and III to yield the net absorbances for the three levels. Use the temperature dependence equation and coefficients given above to correct the measured net absorbance values from the temperature recorded in Step 4 to the certification temperature of  $22^\circ\text{C}$ . The temperature-corrected net absorbances should agree with the certified values within a tolerance determined by overlap of the certified uncertainties and the uncertainty of the user's experimental method. The latter uncertainty may typically include replication uncertainty (if determined), the cuvette pathlength uncertainty, and the specified accuracy uncertainty for the spectrophotometer. Consult the manufacturer of the instrument if adequate agreement is not achieved.
- Step 6. Upon completion of measurements, empty the cuvettes and any material remaining from the four ampoules into a container for proper disposal.

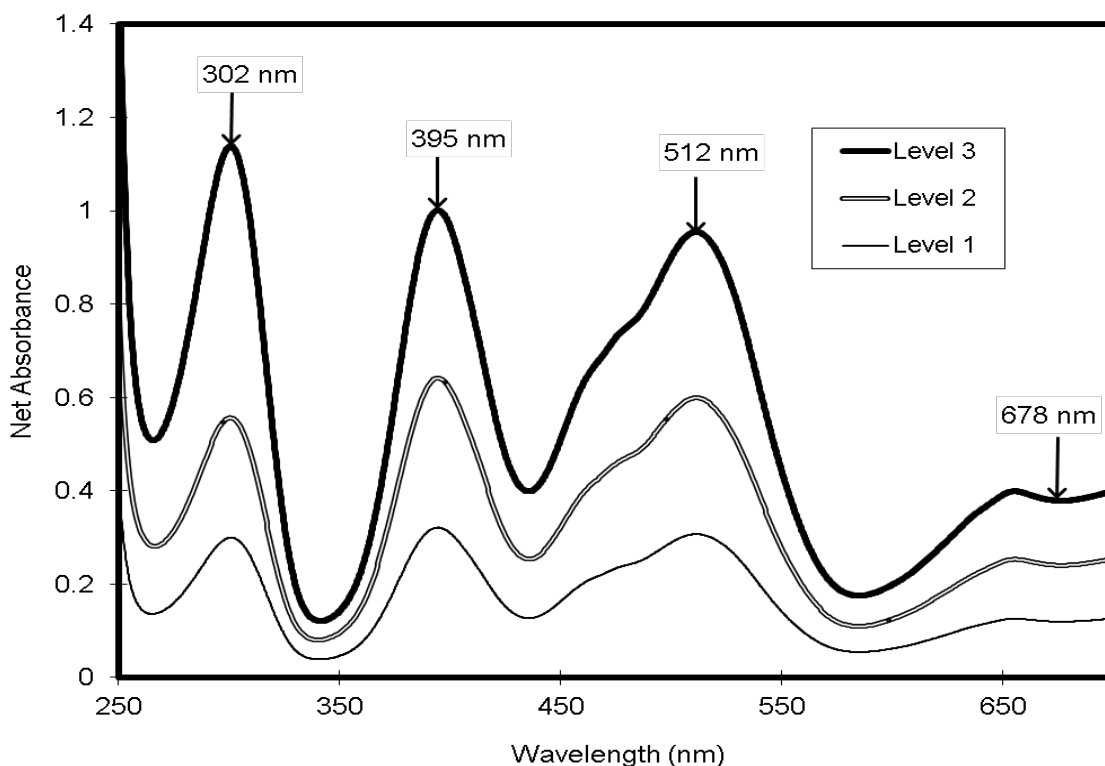


Figure 1. Absorbance Spectrum of SRM 931h

## REFERENCES

- [1] Burke, R.W.; Deardorff, E.R.; Menis, O.; *Liquid Absorbance Standards*; J. Res. Natl. Bur. Stand., Vol. 76A, pp. 469–482 (1972).
- [2] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at [http://www.bipm.org/utls/common/documents/jcgm/JCGM\\_100\\_2008\\_E.pdf](http://www.bipm.org/utls/common/documents/jcgm/JCGM_100_2008_E.pdf) (accessed Mar 2016); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at <http://www.nist.gov/pml/pubs/index.cfm> (accessed Mar 2016).
- [3] Mavrodineanu, R.; *An Accurate Spectrophotometer for Measuring the Transmittance of Solid and Liquid Materials*; J. Res. Natl. Bur. Stand., Vol. 76A, pp. 405–425 (1972); see also *Accuracy in Spectrophotometry and Luminescence Measurements*; NBS Special Publication 378, Mavrodineanu, R.; Shultz, J.I.; Menis, O.; Eds.; U.S. Government Printing Office: Washington, DC (1973).

*Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*