U.S. Department of Commerce
Juanita M. Kreps
Secretary
National Bureau of Standards
Ernest Ambler, Acting Director

National Bureau of Standards Certificate

Standard Reference Material 931b

Liquid Absorbance Standards for Ultraviolet and Visible Spectrophotometry

R. W. Burke and E. R. Deardorff

This Standard Reference Material is certified as solutions of known net absorbance at specific spectral wavelengths. It is intended primarily for use in the calibration and checking of accuracy of the photometric scale of narrow bandpass spectrophotometers employed in clincial analysis and for routine critical evaluation of daily working standards used in clinical spectrophotometry. This Standard Reference Material is applicable for calibrating those instruments that provide an effective spectral bandpass of 1.5 nm or less at 302 nm, 2.0 nm or less at 395 nm, 3.3 nm or less at 512 nm and 8.5 nm or less at 678 nm [1].

Filter	Net Absorbance[a] Wavelength and (Bandpass), nm			
	302(1.0)	395(1.7)	512(2.0)	678(6.5)
"I" - "Blank"	0.306 ± 0.003	0.302 ± 0.003	0.300 ± 0.003	0.115 ± 0.002
"II" - "Blank"	.607 ± .004	.605 ± .004	.602 ± .004	.230 ± .003
"III" - "Blank"	.891 ± .005	.902 ± .005	.899 ± .005	.342 ± .003

[a]Net absorbances ("I" - "Blank", "II" - "Blank", and "III" - "Blank") were determined using 10.00 mm cuvettes (SRM 932) at 25 °C. See Instructions for Use.

Absorbance measurements were performed on a high precision double-beam spectrophotometer equipped with a double monochromator. The accuracy of the photometric scale of this instrument was established with the NBS high-accuracy spectrophotometer described by Mavrodineanu [2]. The uncertainties of the certified values include all known sources of possible systematic error and the 95 percent confidence level for the mean.

While no long-term stability studies have been made on this lot (931b), studies on previous lots (931 and 931a) over a three-year period showed no degradation of the material when stored in the original sealed ampoules. Nevertheless, until additional information is forthcoming, it is recommended that this material not be used after three years from the date of purchase.

The overall direction and coordination of technical measurements leading to certification were performed under the chairmanship of I. L. Barnes.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by W. P. Reed.

Washington, D.C. 20234
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J. Paul Cali, Chief
Office of Standard Reference Materials

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Temperature Dependence

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

$$A_t = A_{25}[1+C_A(t-25)],$$

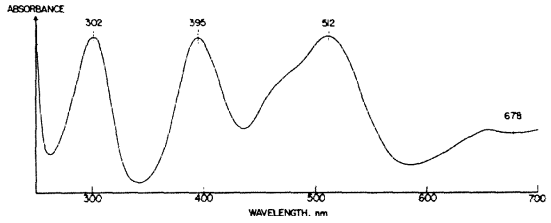
where: A_t = Absorbance at temperature t (°C)
A_{25} = Absorbance certified at 25.0 °C
C_A = Fractional change in absorbance per °C

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

Preparation of Filters

The filters were prepared by dissolving high-purity cobalt and nickel in a mixture of nitric and perchloric acids. The absorbance spectrum of the resulting solution is shown in the following figure. The maxima 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.



Warning

This Standard Reference Material is intended for "in vitro" diagnostic use only.

Instructions for Use

This material is for use as a spectrophotometric absorbance standard.

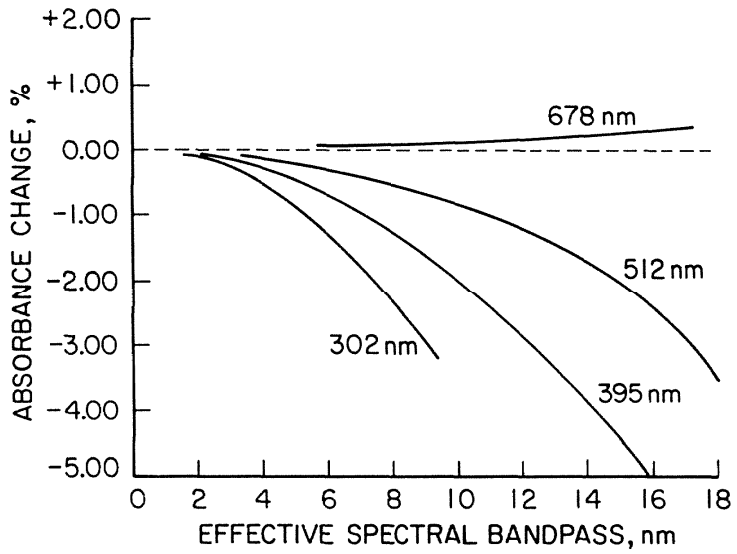
1. Select two clean 10.00 mm cuvettes free of scratches. At least one should be fitted with a ground glass or Teflon stopper to minimize evaporation. Reserve it for all sample measurements.
2. Mark each of the cuvettes to assure the same orientation in the spectrophotometer.
3. Place the cuvettes in 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 spectral bandpass limitations given on the face of the certificate.
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", "I", "II", and "III" against distilled water. Shake each ampoule before opening to remix any condensate which may have 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.

The above instructions 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.

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. 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 insure that the measured absorbances are not significantly different from the certified values, the following restrictions are placed on the size of the spectral bandpass selected: To obtain ±0.1 percent of the true value, 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. For ±0.2 percent, the respective bandpasses should not exceed 2.2, 2.9, 4.8, and 12.3 nm. Additional information on the effect of spectral bandpass on the absorbances of these filters is given in the figure below. These curves are not to be used, however, to correct the measured absorbances.



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. In addition, it is recommended that this Standard Reference Material not be used after three years from the purchase date.

References

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

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

Note: The above papers 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. Government Printing Office, Washington, D.C. 20402, 1973.