

National Bureau of Standards

Certificate

Standard Reference Material 938

4-Nitrophenol

This Standard Reference Material (SRM) is intended primarily for use in calibrating spectrophotometers for clinical analyses in which 4-nitrophenol is the chromogenic compound that is formed.

The certified apparent* specific absorbance, ϵ_a , at 401 nm and 23.5 °C is $131.48 \pm 0.33 \text{ L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$, for solutions containing between 4 and 5 $\text{mg}\cdot\text{L}^{-1}$ of the SRM in aqueous 0.010 $\text{mol}\cdot\text{L}^{-1}$ sodium hydroxide. ("Specific absorbance" is defined here as absorbance per unit pathlength and unit concentration. The term "absorptivity" was avoided because it is ambiguously defined. See Ref. 1.)

The uncertainty given includes the random errors of the measurement process as well as estimates of the inaccuracy of the spectrophotometer and of the inhomogeneity of the SRM.

For solutions prepared at 23.5 °C and measured at higher temperatures (up to 38 °C), the specific absorbance will be higher than the certified specific absorbance by $0.067 \text{ L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$ per degree Celsius.

The material was obtained from the Aldrich Chemical Company, Inc., Milwaukee, Wis. Based on titration with alkali, the purity of the material is estimated to be 99.75% by weight. Karl Fischer titration indicated the presence of about 0.07% water. Paired-ion liquid chromatography showed no 2-nitrophenol or 3-nitrophenol at a detection limit of 1 part per 1000. (For further information on the techniques used, see Ref. 2.) The chemical identity of the material was confirmed by nuclear magnetic resonance spectroscopy. (See Section on Supplementary Information.)

*The term "apparent" is used because no corrections have been applied to the data for the effects of internal multiple reflections within the cuvette or for buoyancy, i.e., the weights used to express concentrations have not been corrected to vacuum. These combined corrections do not exceed 0.2 percent.

NOTICE AND WARNINGS TO USERS

This SRM is intended for "in vitro" diagnostic use only.

STORAGE: SRM 938 should be stored in the original low-actinic glass bottle at room temperature (30 °C or less). It should not be exposed to heat, moisture, or direct sunlight during storage. Refrigerated storage is recommended. However, the material should be allowed to warm to room temperature before opening the container. Under proper storage, this material should be stable for at least five years. If the purity of the material degrades beyond the limits certified, purchasers will be notified by NBS. SRM 938 is not certified for use after five years from the date of purchase.

Analyses leading to certification were performed by J. Baldwin, R. Botto, R. Burke, R. Christensen, B. Coxon, and R. Schaffer.

Statistical analysis of the data was done by J. Mandel and R. Paule.

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

Washington, D.C. 20234
May 15, 1981

George A. Uriano, Chief
Office of Standard Reference Materials

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Instructions for Use

This material is not hygroscopic under ordinary conditions of storage and use; it may be used without preliminary drying. Drying at elevated temperatures or the crushing or grinding of the crystals has been found to cause some loss of water and therefore should be avoided.

The following procedure was found to be satisfactory for the preparation of stock and working solutions. Weigh approximately 140 mg of the SRM to the nearest 0.01 mg and transfer to a dry, one-liter flask weighed to the nearest 0.1 g. Add approximately one kg of either Type I or Type II Clinical Laboratory Reagent Water (3), swirling the flask to dissolve the crystals, and weigh flask and its contents to the nearest 0.1 g.

The dilution of the stock solution to prepare the working solution is performed as follows: Use a plastic syringe as a weight-burette to weigh a 30 to 35 mL-aliquot of the stock solution to the nearest 0.1 mg. Transfer to a one-liter volumetric flask and reweigh the syringe. Add 10 mL of 1.0 mol·L⁻¹ aqueous sodium hydroxide by pipet, and fill the flask to the mark with the water. Prepare the blank solution by using the same sodium hydroxide solution and water.

Supplementary Information

Material Testing:

The lot of material was obtained in ten bottles. Inter-bottle homogeneity was tested by titrating a sample from each bottle with alkali and by determining the moisture content of an additional sample from each bottle by the Karl Fischer procedure. No significant differences among samples were observed with either of these tests.

All transmittance measurements were performed against air on the NBS high accuracy spectrophotometer located in the Center for Analytical Chemistry. The design and construction of this instrument has been described previously [4]. Its accuracy was verified using the double aperture radiation addition principle. All measurements were performed at 23.5 °C and at a spectral bandpass of 0.8 nm.

The apparent specific absorbance, ϵ_a , was calculated from the relationship:

$$\epsilon_a = \frac{D_s - D_b}{b \times c} = \frac{A_a}{b \times c}$$

where:

- ϵ_a = apparent specific absorbance
- A_a = apparent absorbance
- D_s = transmittance density of the sample solution, $-\log_{10} T_s$
- D_b = transmittance density of the blank solution, $-\log_{10} T_b$
- b = internal cuvette pathlength, cm
- c = concentration of 4-nitrophenol solution, g·L⁻¹

The chemical identity of the material was confirmed by pulsed, Fourier-transform proton, carbon-13, and nitrogen-15 nuclear magnetic resonance (NMR) spectroscopy. Proton NMR spectroscopy was performed at 400 MHz (5) using a solution of 202 mg of SRM 938 in 0.6 mL dimethyl sulfoxide- d_6 (d, 100 atom %) containing tetramethylsilane (TMS) as an internal reference. The 4-nitrophenol was characterized by the observation of a singlet (integral 1) at 11.12 ppm and identical sextets (integral, 2 each) at 8.16 and 7.00 ppm due to the symmetrical AA'XX' spin system of the aromatic protons.

Carbon-13 NMR spectroscopy was conducted at 22.6 MHz using a solution containing 205 mg of SRM 938 in 1.0 mL dimethyl sulfoxide- d_6 and TMS as an internal reference. The proton decoupled, carbon-13 NMR spectrum displayed singlet resonances of appropriate intensities at 164.0 (C-1), 139.8 (C-4), 126.1 (C-3 and C-5), and 115.8 ppm (C-2 and C-6).

For nitrogen-15 NMR spectroscopy at 40.5 MHz (5), a solution of 1.08 g of SRM 938 in 3.0 mL of dimethyl sulfoxide- d_6 was used together with a capillary of aqueous, saturated $\text{NH}_4^{15}\text{NO}_3$ solution as an external reference. The nitrogen-15 signal of the nitro group of the 4-nitrophenol was observed as a singlet at -5.62 ± 0.02 ppm. A nitrogen-14 chemical shift of -7 ± 1 ppm in acetone solution has been reported (6).

References

1. Mielenz, K. D., *Anal. Chem.* **48**, 1093-1094 (1976).
2. Bowers, G. N., Jr., McComb, R. B., Christensen, R. G. and Schaffer, R., High Purity 4-Nitrophenol. Purification, Characterization and Specifications for Use as a Spectrophotometric Standard Reference Material, *Clin. Chem.* **26**, 724 (1980).
3. Specifications for Reagent Water Used in the Clinical Laboratory NCCLS Approved Standard: ASC-3, National Committee for Clinical Laboratory Standards, Villanova, Pa., 1980.
4. Mavrodineanu, R., An Accurate Spectrophotometer for Measuring the Transmittance of Solid and Liquid Materials, *NBS Journal of Research* **76A**, No. 5, 405-425 (1972).
5. These NMR spectra were recorded at the high field NMR facility of the National Measurement Laboratory, NBS.
6. Witanowski, M., Stefaniak, L., and Webb, G.A., *J. Chem. Soc. B*, 1065 (1967).

This Standard Reference Material has been measured and certified at the laboratories of the National Bureau of Standards, Gaithersburg, Maryland. All inquiries should be addressed to:

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