Application Note

Environmental

Agilent

Trusted Answers

SPANDS Colorimetric Method to Quantify Fluorides in Water

Convenient and precise concentration measurements with the Agilent Cary 60 UV-Vis spectrophotometer

Authors

Rogelio García and
Geethika Weragoda
Agilent Technologies, Inc.

Abstract

The Agilent Cary 60 UV-Vis spectrophotometer was used to quantify fluoride in water as described in the SPANDS colorimetric method. The Concentration module in Agilent Cary WinUV software was used for single wavelength absorbance measurements and data analysis, reducing time-consuming data workup procedures. The calibration curve has a linear analytical range between 0 to 1.4 mg/L, which can be used in analyzing water samples.

Introduction

Fluoride ions are found naturally in water. Both surface and underground water contain fluoride that occurs naturally from weathering of rocks and soils that contain fluoride compounds. In 2011, the World Health Organization (WHO) suggested a safe level of fluoride of 0.5 to 1.5 mg/mL, which depends on climate, environment, and other sources of fluoride.[1] Fluoride is typically added to public drinking water sources in many places around the world. This controlled adjustment of fluoride content in drinking water is called fluoridation and is considered to be a cost-effective way to deliver fluoride to the community. Some groundwater and natural springs can have naturally high levels of fluoride. Prolonged exposure to excessive amounts of fluorides may also lead to a wide range of adverse health effects, including fluorosis, cancer, impaired brain development, and many more.[2] Thus, accurate determination of fluoride content in drinking water has received greater attention as an important public health measure.

Different methods of quantifying fluorides in water are explained in the Environmental Protection Agency (EPA) reference method, Standard Method 4500-F. Among these, the SPANDS colorimetric method (method D) is considered the most accepted method, with a linear analytical range of 0 to 1.4 mg/L. However, the use of nonlinear calibration can extend the analytical range up to 3.5 mg/L. Under acidic conditions, the zirconium-SPANDS dye is dissociated by the fluoride ions to generate colorless complex anions (ZrF_6^{-2}) and yellow SPANDS, thereby discoloring (bleaching) the red color of the dye. This discoloration reaction can be monitored by UV-Vis spectroscopy at 570 nm. Since discoloration is a function of fluoride ions and directly proportional to the concentration of fluoride, it is used to quantify fluorides in water. The corresponding quantification method is created using a standard calibration curve plotted against absorbance measurements taken at 570 nm for a series of standard fluoride samples with different fluoride concentrations.

In this application note, the Agilent Cary 60 UV-Vis Spectrophotometer, and the Concentration module in the Agilent Cary WinUV software, were used to create a method to quantify fluorides in water with reference to the SPANDS colorimetric method. When analyzing an unknown sample, the software automatically uses the calibration curve to calculate and report the sample concentration, reducing time-consuming data workup procedures. The Cary WinUV software can be tailored to fit analytical requirements and to simplify UV-Vis measurements to get more done in less time. The Cary WinUV software includes powerful features and streamlined methods for data collection, analysis, storage, and display, while reducing complexity. It also includes different modules that are designed to cover a range of applications including qualitative wavelength scans or reads, concentration analysis, enzyme kinetics, and many more.

Figure 1. The Agilent Cary 60 UV-Vis spectrophotometer.

Experimental

Instrumentation

The Cary 60 UV-Vis spectrophotometer and a quartz 10 mm pathlength cell were used in this study. Data acquisition was carried out using the Concentration module of the Cary WinUV software, version 5.1.3.1042. The single wavelength absorbance measurements were collected at 570 nm in three replicates for each sample using the parameters given in Table 1, and the corresponding calibration curve was generated automatically by the software. The fit type for the calibration curve as well as minimum R^2 (a measure for the fit quality) can be set by the operator.

Table 1. Experimental parameters.

Parameters	Setting
Wavelength (nm)	570
Signal Averaging Time (s)	0.1
Replicates	3
Fit Type	Linear
Min R^2	0.95000

Materials and sample preparation

- **Zirconyl-acid reagent**: 133.0 mg of $ZrCl_2{\cdot}8H_2O$ was dissolved in 25 mL of distilled water. 350 mL of concentrated HCl acid was added and diluted up to 500 mL with distilled water.
- **SPANDS solution**: 958.0 mg of SPANDS was dissolved in distilled water and diluted up to 500 mL with distilled water to prepare a red solution. The solution was stored in an amber bottle to protect it from sunlight.
- **Acid zirconyl-SPANDS reagent**: Equal volumes of SPANDS solution and zirconyl-acid reagent were mixed to prepare a red solution.
- **Reference solution**: 10 mL of the SPANDS solution was diluted up to 100 mL with distilled water. 7 mL of concentrated HCl was diluted to 10 mL using distilled water. 10 mL of the acid solution was then added to the previously diluted SPANDS solution. The resulting solution was mixed and used to adjust the reference point (zero) of the spectrophotometer.
- **Standard fluoride solutions for the calibration curve**: All standard fluoride solutions were prepared in polyethylene flasks and stored in polyethylene bottles, since the fluoride ions attack glass.
- **Stock fluoride solution**: 221.0 mg of anhydrous sodium fluoride was dissolved in distilled water and diluted to 1,000 mL with distilled water (100 mg/L).
- **Standard fluoride solution**: 100 mL of stock fluoride solution was diluted to 1,000 mL with distilled water (10 mg/L).
- **Standard fluoride solutions**: A series of standard fluoride solutions with concentrations 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/L were prepared by diluting appropriate quantities of standard fluoride solution to 50 mL with distilled water. 10 mL of mixed acid-zirconyl-SPANDS solution was added to each standard solution and mixed well to prepare a colored solution for the photometric measurements.

Creating a fluoride quantification method

The fluoride quantification method was created using the Concentration module provided within the Cary WinUV software. The method setup consists of two steps: (1) Create a method to measure the standard samples and (2) Measure the standard solutions and create the quantification method for the analysis of samples.

1. Create a method to measure the standard samples.

Creating a method in the Cary WinUV Concentration module is quick and easy, requiring a few simple steps as follows and as illustrated in Figure 2:

A. Open the Concentration module and click the **Setup** tab to open the instrument setup window.

B. In the Cary tab, enter the wavelength for single wavelength measurements in the Wavelength option (In this example, enter 570 nm).

C. Enter the number of replicates that are needed for each standard using **Replicates** or **Sample/Std Averaging**. In this example, data were collected for each standard solution in three replicates.

D. In the Standards section, enter concentrations of standard samples in ascending order and select the **Fit type**. In this example, Fit type was selected as Linear for a concentration range of 0 to 1.4 mg/L.

Note: The SPANDS colorimetric method provides a linear analytical range of 0 to 1.4 mg/L. However, the concentration range could be extended up to 3.5 mg/L using a nonlinear calibration. Simply select the fit type as Quadratic for an extended concentration range.

- Both the software and the instrument are now ready. Click the **Start** button to start the measurement of the standard solutions.

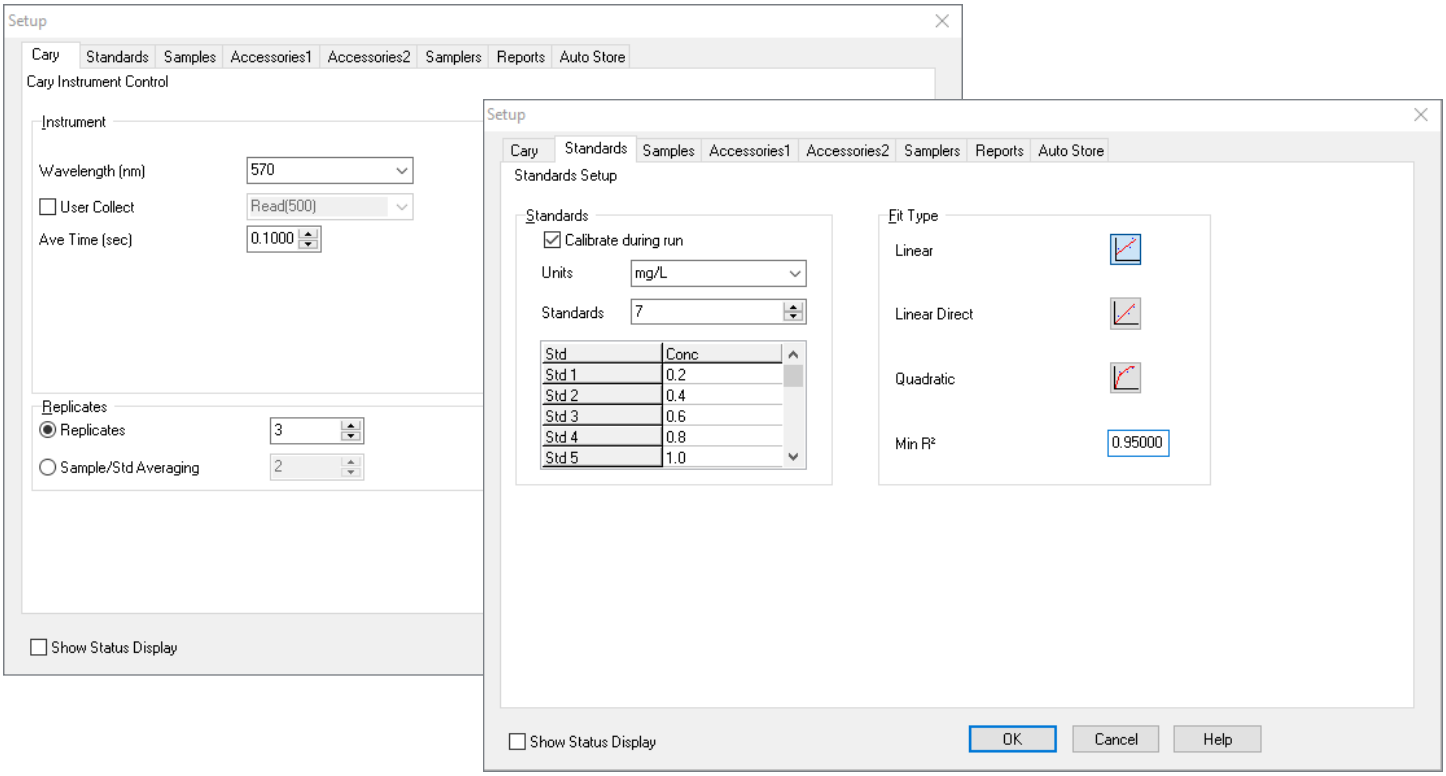

Figure 2. Instrument setup for data acquisition and analysis using the Concentration module of the Agilent Cary WinUV software.

2. Measure standard solutions and create the quantification method.

Click the **Start** button and follow the software instructions to begin the analysis. First, the Cary UV-Vis spectrophotometer was set to zero absorbance using the reference solution. The single wavelength absorbance measurements for standard samples were then collected at 570 nm by simply following the sample loading instructions in the Cary WinUV software. Following the data acquisition, the corresponding calibration curve and concentration analysis reports were automatically generated by the software (Figure 3A). A linear calibration curve with a negative slope of 0.1872 and correlation coefficient (R^2) of 0.9993 were obtained for the concentration range of 0 to 1.4 mg/mL. The concentration analysis report comprises calibration data such as sample concentration, mean absorbance, calibration equation, and correlation coefficient. In addition, the standard deviation (SD) and the percent relative standard deviation (%RSD) of absorbance measurements of each sample are presented in the report for easy data analysis (Figure 3B). The result report can be easily customized with preferred font size, color, and font type. The fluoride quantification method was saved in the software to be reused in analyzing samples.

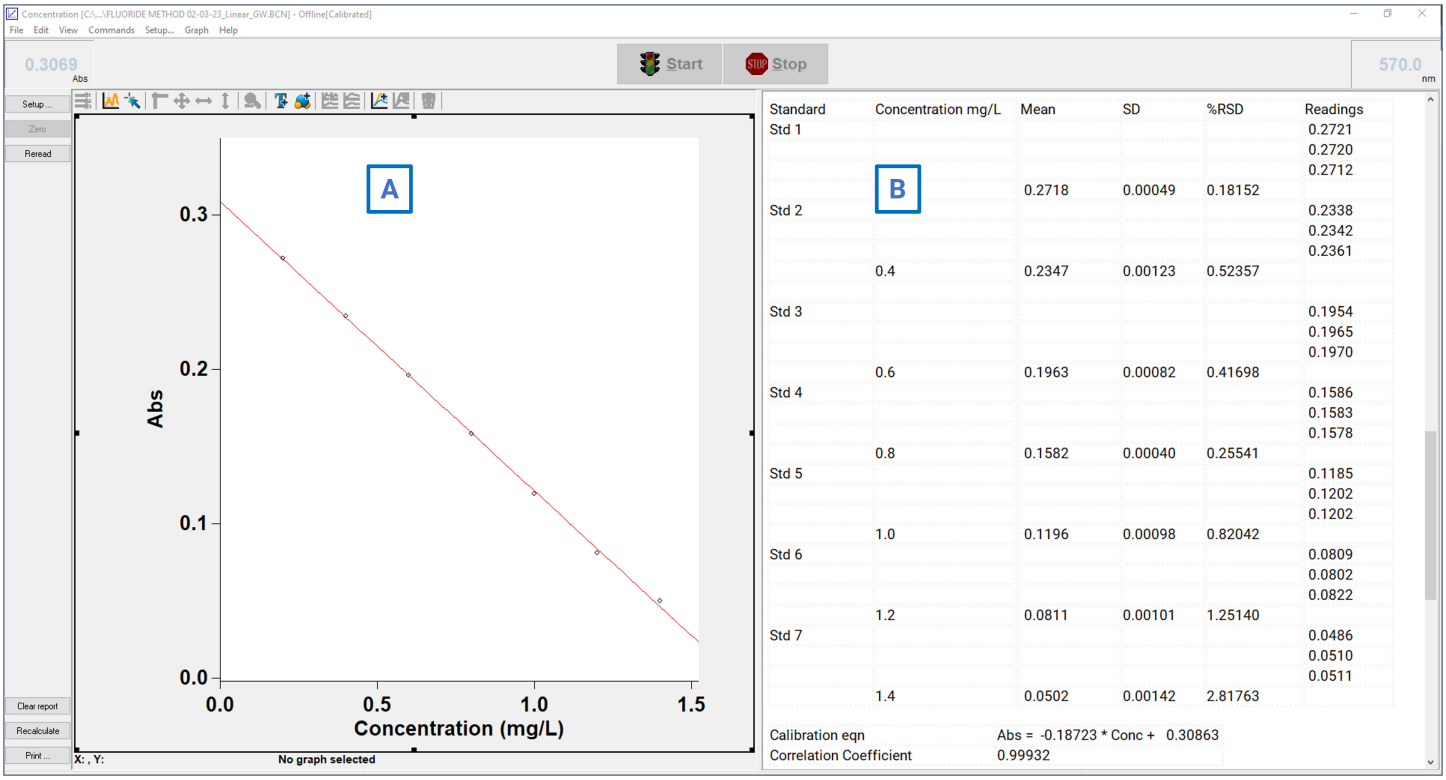

Figure 3. The calibration curve and the concertation analysis report are automatically generated by the Agilent Cary WinUV software.

3. Use quantification method to analyze samples.

Once the quantification method is set up as outlined in the previous section, analyzing an unknown sample takes only a few seconds. To demonstrate its application, internally prepared fluoride samples with known concentrations (0.5 and 0.7 mg/L) were analyzed. Following the single wavelength absorption measurements at 570 nm (in three replicates), the software automatically applied the calibration curve and calculated the fluoride concentration of each sample. The corresponding result report generated by the software includes sample concentrations, absorption values for each scan, mean absorbance, and relative standard deviation (Figure 4).

Calibration eqn Abs = -0.18723 * Conc + 0.30863
Correlation Coefficient 0.99932
Calibration time 5/2/2023 11:33:45 AM

Analysis
Collection time 2/3/2023 3:33:40 PM

Sample	Concentration mg/L	Mean	SD	%RSD	Readings
Sample 1					0.1786
					0.1796
					0.1789
	0.7	0.1790	0.00051	0.28663	
Sample 2					0.1799
					0.1788
					0.1796
	0.7	0.1794	0.00057	0.31690	
Sample 3					0.2164
					0.2177
					0.2155
	0.5	0.2165	0.00111	0.51080	

Figure 4. A snapshot of the concentration analysis report generated automatically by the Agilent Cary WinUV software.

Analyzing water samples using the SPANDS colorimetric method

The analysis of water samples that are free from interfering ions can be performed using the steps described in the previous section.

Sample pretreatment: Interfering ions cause errors, and these can be removed by distillation as explained in EPA Method 340-1. If the water sample contains residual chlorine, it can also be removed by adding one drop (0.05 mL) of $NaAsO_2$ solution, prepared by dissolving 5.0 g of $NaAsO_2$ and diluting to 1,000 mL with distilled water.

Add 10 mL of mixed acid-zirconyl-SPANDS solution into 50 mL of water (free from interfering ions) and mix well to generate a red-colored solution for the analysis. Collect the single wavelength absorbance measurement of the water sample at 570 nm using the quantification method described in the previous section. Following the data acquisition, the software automatically uses the calibration curve to calculate and report the sample concentration. If the sample absorbance falls beyond the analytical range of the calibration curve, which is 0 to 1.4 mg/L, repeat the analysis by diluting the sample.

Conclusion

The Agilent Cary 60 UV-Vis spectrophotometer and the Agilent Cary WinUV software provide a convenient and easy-to-use platform for quantifying fluorides in water as described in the SPANDS colorimetric method. The fluoride quantification method was created using the Agilent Cary WinUV Concentration module. Setting up the software is quick and easy and requires only a few simple steps. The SPANDS colorimetric method has a linear analytical range of 0 to 1.4 mg/L. The quantification method created in the Cary WinUV Concentration module can be directly applied to analyze water samples. The software automatically calculates and reports the sample concentration, reducing time-consuming data workup procedures.

References

1. Guidelines for Drinking-Water Quality, 4th Edition WHO, **2011**.
2. Shahroom, N. B.; Mani, G.; Ramakrishnan, M. Interventions in Management of Dental Fluorosis, an Endemic Disease: A Systematic Review. *J. Family Med. Prim. Care* **2019**, *8(10)*, 3108.
3. Standard Methods 4500-F- A, B and D. Determination of Fluoride F- Spectrophotometric Method; Standard Methods for the Examination of Water and Wastewater, 22nd Edition, American Public Health Association.

Further information

- Agilent Cary 60 UV-Vis Spectrophotometer
- Agilent Cary WinUV Software
- Find Out Which Agilent UV-Vis Is Best for Your Needs
- Agilent UV-Vis Spectrophotometer Uses & Applications
- Agilent UV-Vis Spectroscopy & Spectrophotometer FAQs

www.agilent.com

DE26463005

Printed in the USA, June 1, 2023
5994-6187EN

Agilent
Trusted Answers