24 h of collection. Keep samples cold until analysis, and warm them up to room temperature before measurement.

b. Sample preparation: Check sample pH. If outside the range of 4 to 10, preferably adjust sample to pH 7 and note the adjustment.

If true color is to be measured, wash membrane filter and filter assembly by passing at least 50 mL water through filter. Filter about 25 mL sample and discard filtrate. Filter a further portion of about 50 mL through the same filter and retain for analysis.

c. Sample measurement: Observe sample color by filling a matched nessler tube to the 50-mL mark with sample and comparing it with standards. Look vertically downward through tubes toward a white or specular surface placed at such an angle that light is reflected upward through the columns of liquid. If turbidity is present and has not been removed, report as "apparent color." If the color exceeds 100 units, dilute sample in known proportions until the color is within the range of the standards.

6. Calculation

a. Calculate color units (CU) by the following equation:

$$\text{Color} = \frac{A \times 50}{B}$$

where:

A = estimated color of a diluted sample, and
B = mL sample taken for dilution.

b. The correct units for true color are CU. One CU is equivalent to one Hazen unit and to one Pt-Co unit. If samples are not filtered, report data as Apparent CU. Report color results in whole numbers and record as follows:

CU	Record to Nearest
1–50	1
51–100	5
101–250	10
251–500	20

c. Report sample pH.

7. Quality Control

The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

a. Replicate measurements: Use at least two portions of filtered sample.

b. Duplicate analyses: Analyze every tenth sample in duplicate (i.e., duplicating the entire procedure) to assess method precision.

c. Pre-programmed spectrophotometers: For spectrophotometers with pre-programmed calibration curves, verify calibration curve regularly with the platinum-cobalt standards prepared under 2120C.4, and adjust pre-programmed curves as needed.

8. Reference

1. BLACK, A.P. & R.F. CHRISTMAN. 1963. Characteristics of colored surface waters. *J. Amer. Water Works Assoc.* 55:753.

9. Bibliography

CHRISTMAN, R.F. & M. GHASSEMI. 1966. Chemical nature of organic color in water. *J. Amer. Water Works Assoc.* 58:723.

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2120 C. Spectrophotometric—Single-Wavelength Method (PROPOSED)

1. General Discussion

a. Principle: Color is determined spectrophotometrically at a wavelength between 450 and 465 nm, with platinum-cobalt solutions as standards.[1–3] True color of real samples and platinum-cobalt standards follows Beer's Law.

b. Application: The spectrophotometric platinum-cobalt method is applicable to natural waters, potable waters, and wastewaters, both domestic and industrial.

c. Interference: The primary interference is from the presence of colloidal and suspended particles that absorb or scatter light at the wavelength of the spectrophotometric method. While in 2120B color measurements can be made without removal of particulate matter as long as they are reported as "Apparent CU", 2120C requires removal of particulate matter before color determination.

Light absorbance of organic matter depends on pH; however, the variation in absorbance is small for the pH range of most waters. Because color measurements are made for aesthetic reasons, preferably do not adjust sample pH as long as it is between 4 and 10. If pH is adjusted, adjust to 7, and note. Further, pH can affect the solubility of substances, which can then interfere with the color measurement if particulate matter is formed.

d. Method detection level: The minimum detectable color depends on the cell path length. Choose a cell size that provides an absorbance within the range that results in good accuracy and linearity of response. This range depends on the quality of the spectrophotometer. If a 50-mm cell is used in the wavelength range of 450 to 465 nm, then an absorbance of 0.005 yields a minimum detectable color of 1 CU. With newer spectrophotometers, a method detection level of 2 CU can be obtained with a path length of 25 mm. Dilute samples with high color to fall within the range of the standard curve. Absorbance readings should fall within the range of 0.005 to 0.8.

2. Apparatus

a. Spectrophotometer: Choose a wavelength between 450 and 465 nm. Use matched glass cells providing a light path of at least 25 mm. Cells with path lengths of 40, 50, or 100 mm may be

used. Beer's Law allows flexibility in selecting the cell path length.

b. Filter and filter assembly: See 2120B.2*c*.

3. Reagents

See 2120B.3.

4. Preparation of Standards

Prepare stock color solution of 500 CU according to 2120B.4.

Prepare standards having CU of 5, 10, 15, 20, 30, 40, 50, and 100 by diluting 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, and 20.0 mL stock color standard with water in 100-mL volumetric flasks. Protect standards against evaporation and contamination when not in use. Keep in the dark when not in use, and keep for only 1 month.

5. Spectrophotometric Standard Curve

Let spectrophotometer warm up according to manufacturer's instructions. Choose a wavelength between 450 and 465 nm to develop the standard curve; a good choice is 456 nm. The absorbance of Pt-Co has a broad maximum absorbance within this wavelength range. Use matched spectrophotometer cells. Fill one cell with water to zero the instrument. Read absorbance for each color standard, and prepare a standard curve of CU versus absorbance.

Pre-programmed color curves are available with some spectrophotometers. The curves can be verified by use of the standards prepared in 2120C.4.

6. Procedure

a. Sample collection: See 2120B.5*a*.

b. Sample preparation: See 2120B.5*b*. Always filter sample.

c. Spectrophotometric measurement: Let spectrophotometer warm up according to manufacturer's instructions. Set wavelength at same setting used to develop the standard curve; be sure that the cell path length is the same as that used for the standard curve. Fill one spectrophotometer cell with water and zero the instrument. Rinse the other cell with sample and then refill. Place cell in spectrophotometer and read absorbance. Repeat for remaining samples. Determine sample color using absorbance readings and standard curve relating absorbance and CU. For spectrophotometers with pre-programmed calibration curves for color, zero instrument and take sample measurements according to manufacturer's instructions.

7. Quality Control

See 2120B.7.

8. References

1. Crowther, J. & J. Evans. 1981. Estimating color in Hazen units by spectrophotometry. *J. Amer. Water Works Assoc.* 73:265.
2. Bennett, L. & M. Drikas. 1993. The evaluation of color in natural waters. *Water Res.* 27:1209.
3. Hongve, D. & G. Åkesson. 1996. Spectrophotometric determination of water colour in Hazen units. *Water Res.* 30:2771.

2120 D. Spectrophotometric—Multi-Wavelength Method

1. General Discussion

a. Principle: The color of a filtered sample is expressed in terms that describe the sensation realized when viewing the sample. The hue (red, green, yellow, etc.) is designated by the term "dominant wavelength," the degree of brightness by "luminance," and the saturation (pale, pastel, etc.) by "purity." These values are best determined from the light transmission characteristics of the filtered sample by means of a spectrophotometer.

b. Application: This method is applicable to potable and surface waters and to wastewaters, both domestic and industrial.

c. Interference: The primary interference is from the presence of colloidal and suspended particles that absorb or scatter light.

d. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

2. Apparatus

a. Spectrophotometer, having absorption cells of a minimum of 10 mm, a narrow (10-nm or less) spectral band, and an effective operating range from 400 to 700 nm.

b. Filter: See 2120B.2*c*.

3. Procedure

a. Sample preparation: Bring two 50-mL samples to room temperature. Use one sample at the original pH; adjust pH of the other to 7.0 by using sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) of such concentrations that the resulting volume change does not exceed 3%. A standard pH is necessary because of the variation of color with pH. Remove particulate matter from samples before color determination (see 2120B.5*b*).

b. Determination of light transmission characteristics: Thoroughly clean 1-cm absorption cells. Rinse twice with filtered sample, and fill cell with filtered sample.

Determine transmittance values (in percent) at each visible wavelength value presented in Table 2120:I, using the 10 ordinates marked with an asterisk for fairly accurate work and all 30 ordinates for increased accuracy. Set instrument to read 100% transmittance on the distilled water blank and make all determinations with a narrow spectral band.