Uses for a Holmium Oxide Filter in Spectrophotometry

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The use of a holmium oxide calibration filter is discussed for checking wavelength, trouble-shooting, and adjusting sensitivity of spectrophotometers. Correction tables may be prepared to permit setting the instrument to true wavelength. One spectrophotometer was found to have a wavelength error large enough to impair its usefulness in direct spectrophotometric methods.

THE TECHNIC of direct spectrophotometry has elicited much interest because of the constant introduction of new procedures for the analysis of constituents of blood and urine. These include methods for bilirubin (*1–3*), blood oxygen (*4, 5*), uric acid (*6*), porphyrin (*7*), barbiturates (*8*), and many others. The nature of the technic imposes strict requirements on the instrument being used. If the instrument is known to be reliable and meets relatively high specifications, most workers will accept it as being without error. However, if the wavelength calibration is incorrect, direct spectrophotometry will lead to erroneous results, or they may not even be practical. This is particularly true if the error places the observed absorption peak on the ascending or descending slope of the true peak. Thus it is imperative that the wavelength calibration of an instrument be checked not only once, but at frequent intervals thereafter.

A calibration standard made of holmium oxide glass has recently been introduced for checking wavelength calibrations of spectrophotometers.* It is inserted in a metal block which in use replaces the 10-mm. square cell of the instrument. Included with the standard is a spectral transmittance curve determined on a Bausch & Lomb 505 spectrophotometer using a 0.5-mμ band pass. A spectral transmit-

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tance curve run on a new Beckman model DB spectrophotometer validated the curve supplied with the holmium oxide filter.

This spectral transmittance curve shows nineteen well-defined peaks over the range of 280 to 550 mμ. The filter permits the wavelength calibration of an instrument to be checked over a fairly wide range, and also suggests some further uses which will be described here. Sodium or mercury lines may also be used for wavelength calibrations, but this is not a convenient procedure and is frequently beyond the capabilities of the laboratory.

Methods and Results

The holmium oxide filter was used to check the wavelength calibration of several Beckman spectrophotometers in this laboratory (three model DU's and one model DB. The three DU's varied in age from one to eleven years. Figure 1 illustrates the transmittance curve obtained on one of these instruments. The figures in parentheses are the true wavelength which should have been obtained. The error ranged from +4 mμ at 334 mμ to +20 mμ at 536 mμ. When the true wavelength was plotted against the wavelength shown on the instrument dial the variation in error was found to be linear over the range of the spectral scan (Fig. 2). Using this chart, an operator is able to set the instrument at

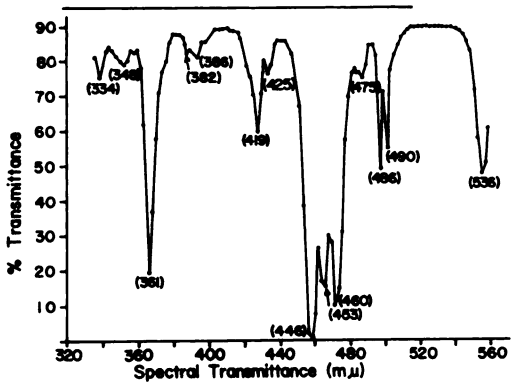

Fig. 1. Spectral transmittance scan with holmium oxide filter in Beckman DU spectrophotometer. Parentheses indicate true wavelength.

the true wavelength: for example, if the analytical procedure required a wavelength of 420 mμ the instrument would be set on 429 mμ.

The other two DU's had maximum wavelength errors of not over 4 mμ. These instruments would require wavelength correction only for those procedures having very narrow absorption peaks.

Discussion

The calibration and correction procedure described illustrates one use of the holmium oxide calibration standard. The error demonstrated in three instruments emphasizes the importance of checking wavelength calibration. This requirement does not imply criticism of

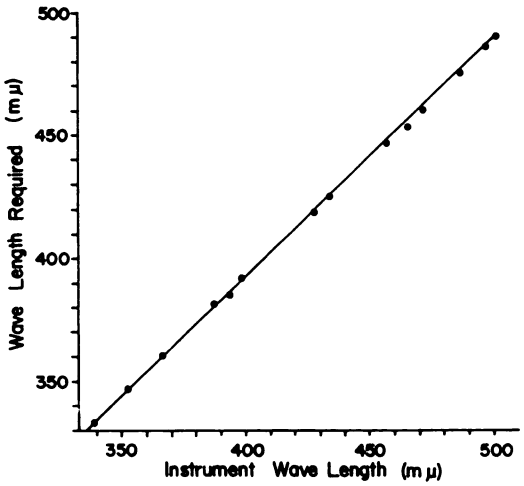

Fig. 2. Graph for correcting instrument (wavelength) setting to true wavelength.

the quality of the analytical instrument. It does serve to demonstrate, however, that even on an instrument of high quality, wavelength calibration can change with time and use. The correction chart enables the operator to obtain the desired wavelength without extensive repair work on the instrument.

The holmium oxide calibration standard has been useful in two other ways. The model DB in our laboratory was found to be correct in its wavelength calibration. Recently we had reason to question the sensitivity of the instrument when using the narrow slit program for a spectral scan. The calibration standard was used for a spectral scan, and this showed poor resolution of the peaks which had previously been well defined. By reverting to a manual slit adjustment, it was possible to use the instrument until the narrow slit program defect had been corrected. Thus the calibration standard can provide a convenient reference when trouble-shooting an instrument.

A third use of the calibration standard has also proved helpful. There are occasions when the maximum sensitivity of an instrument is required. In order to obtain maximum sensitivity the slit width is narrowed as far as possible, and compensation made with increasing gain.

However, it is possible to increase the sensitivity beyond that required for the analysis, thus obtaining unnecessary unsteadiness due to the high degree of amplification involved. The use of the holmium oxide calibration standard as a sensitivity reference permits setting the slit as wide as required to obtain the degree of sensitivity needed without increasing the amplification beyond what is absolutely necessary.

A didymium filter can also be used for the applications described above.* This filter covers a wider spectral range (to 800 mμ), but does not have as many well-defined peaks as the holmium oxide filter in the area 340–600 mμ. Only three peaks occur above 600 mμ. The didymium filter can serve as a useful cross-check on the holmium oxide filter.

Summary

A holmium oxide calibration standard has been used to check several spectrophotometers. Error in wavelength settings was calculated, and a correction graph prepared to convert the setting on the instrument dial to the true wavelength. This filter is also useful in trouble-shooting an instrument, and in adjusting the instrument to a required degree of sensitivity.

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