SPECTROPHOTOMETRIC MEASUREMENTS AGAINST AIR AS A REFERENCE

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In ultraviolet and visible spectrophotometry, it is customary to arrange that the instrument reads zero absorbance (100% transmittance) with a reference cell containing solvent or a 'reagent blank' in the path of radiation and then to replace the reference cell by a cell containing the sample and measure the absorbance (A_1). Immediately before or after these operations, a similar measurement should be made (A_2) with the same liquid (for example, the solvent) in both the cells. The absorbance of the sample is calculated as $A = A_1 - A_2$.

The procedure would be simpler, quicker and cheaper if air were to replace the reference cell containing liquid, but it is important to know whether such a change of technique would affect the accuracy (closeness to the true absorbance of the sample) and the precision (reproducibility) of the measurement of A.

Theoretically the technique of measurement against air should give an identical value for A but the reproducibility, measured as the relative standard deviation (that is, the coefficient of variation), may be expected to increase slightly, as shown below.

Let T represent the transmittance; $A = -\log T$. Let s_T represent the standard deviation of T. Assume that A is directly proportional to the concentration (C) of the absorbing material in the sample. Let c.v. of C represent the coefficient of variation of C. Let r represent the correlation coefficient of T_1 and T_2.

For single measurements of absorbance, c.v. of $C = \dfrac{s_T/T}{2{\cdot}303A}$.

For measurements of the difference between two absorbances ($A = A_1 - A_2$),

$$\text{c.v. of } C = \frac{\left[(s_{T_1}/T_1)^2 + (s_{T_2}/T_2)^2 - 2r(s_{T_1}/T_1)(s_{T_2}/T_2) \right]^{\frac{1}{2}}}{2{\cdot}303\ (A_1 - A_2)}.$$

Several hundred measurements of both A and T of various acidic potassium dichromate solutions at different values of A at one wavelength (350 nm) against a reference cell containing water and against air in a Unicam SP500 spectrophotometer indicate that s_T is approximately independent of T and that r is about 0·85. It appears that there is little difference in precision if T is measured rather than A.

It is well known that for single measurements of absorbance, c.v. of A (which is directly proportional to c.v. of C) has a minimum value at A = 0·434 (Twyman and Lothian, 1933); it is only about 10% greater for values of A from 0·3 to 0·6. For measurements of the difference between two absorbances, with r = 0·85, the minimum value of c.v. of C is at A = 0·302, whatever the individual values of A_1 and A_2, and it is only about 10% greater for values of A from 0·2 to 0·5.

At fixed A, c.v. of C increases as A_2 increases. A_2 is close to zero in the conventional technique of measurement against a reference cell containing solvent, with subtraction of a 'cell blank'. When measurement is made against air, the value of A_2 is in the range 0·03 to 0·05 for common solvents in glass or silica cells; the resulting relative increase in c.v. of C is about 10%.

We recommend that analysts should seriously consider the advantages of measuring absorbances (A_1 and A_2) against air rather than against a reference cell containing a liquid. The result ($A_1 - A_2$) is the same and the coefficient of variation of absorbance (and therefore of concentration) is increased only a little.

Twyman, F. and Lothian, G. F. (1933). Proc. phys. Soc., 45, 643-659.