

Reproducibility of extinctions measured on the slopes of absorption curves

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Errors in setting the wavelength scale of an ultra-violet spectrophotometer cause an increase in the variance of extinctions measured on the slope of an absorption curve. For a given instrument and operator, the coefficient of variation of extinctions measured on a steep slope was ten times the normal value for zero slope. A method is described for obtaining reasonably precise estimates of $(\delta \log E / \delta S)$, where "S" refers to the displacement of the wavelength scale. The coefficient of variation bears a linear relationship to $(\delta \log E / \delta S)$, which is relevant to the choice of wavelengths for Vierordt's method.

WHEN measuring the extinction at a wavelength of maximum or minimum absorption, a small error in setting the wavelength scale or a small change in the spectrophotometer's wavelength calibration, such as may occur with time, produces a negligible effect on the measured extinction. However, as is well known, the situation is very different for measurements on the slope of an absorption curve, which are made frequently in multicomponent spectrophotometric analysis. The reproducibility of this technique may in fact be more dependent upon wavelength setting than upon photometric errors (von Halban & Ebert, 1924; Twyman & Lothian, 1933).

In the course of an experimental evaluation of the precision of Vierordt's method (Glenn, 1960), it proved necessary to develop a weighting system, which would account for the difference between assays in the matter of "wavelength setting errors." An experimental study of the dependence of such errors upon the slope of the absorption curve was essential to this purpose. Hence, for different values of the slope $(\delta \log E / \delta \lambda)$, it was necessary to obtain estimates of $c.v.(\lambda E)$, the coefficient of variation* of extinction measurements which include wavelength setting errors in addition to the inevitable photometric error. The results are also of general interest in view of the dearth of experimental information relating to optimum conditions of spectrophotometric analysis (Crawford, 1959).

$c.v.(\lambda E)$ was obtained from eight successive extinction measurements, each of which entailed a prior re-setting of the wavelength scale. Chlorocresol in 0.1N aqueous H_2SO_4 provided a suitable system of measurement, which readily lent itself to the choice of a set of wavelengths, for which the slopes ranged from "gentle" to "very steep." In view of the decision to study $c.v.(\lambda E)$ as a function of $(\delta \log E / \delta \lambda)$, this choice was made from a graph of $\log E$ against wavelength.

The wavelength setting errors of a careful observer arise from minute variations in the setting of the wavelength scale, so that graphical estimation of slopes from an absorption curve determined at 2 or 3 $m\mu$

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* Throughout the paper, "coefficient of variation" denotes 100 (standard deviation/mean).

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intervals would have been grossly inadequate for the present quantitative study. It was therefore necessary to use a device (Fig. 1), which gave a 20-fold magnification of the wavelength scale displacement. A further problem arose from a characteristic of the prism monochromator, whereby

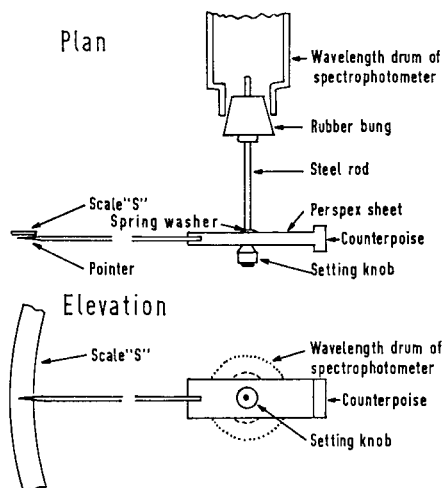


FIG. 1. Device for obtaining a magnified indication of the setting of the wavelength scale.

the wavelength scale becomes increasingly cramped in going from smaller to greater wavelengths. Thus, as can be seen from Table 1, a given wavelength change involves a scale displacement at 220 $m\mu$, which is more than three times that at 300 $m\mu$. Hence, the slope of an absorption curve can be expressed either as $(\delta \log E / \delta \lambda)$, the change in $\log E$ for a given change of wavelength, or $(\delta \log E / \delta S)$, the change in $\log E$ for a given displacement of the wavelength scale. The operator's error in setting the scale to a given mark, should be independent of wavelength, so that $c.v.(\lambda E)$ ought to bear a more simple relationship to $(\delta \log E / \delta S)$ than to $(\delta \log E / \delta \lambda)$.

Mean values of $c.v.(\lambda E)$ were determined at seven chosen wavelengths in a sequence of 29 blocks and the results recorded in Table 2 together

TABLE 1. MEAN VALUES OF $(\Delta \lambda / \Delta S)$ FOR THE UVISPEK PHOTOELECTRIC SPECTROPHOTOMETER

λ_m ($m\mu$)	$\Delta \lambda$ ($m\mu$)	ΔS (cm)	$(\Delta \lambda / \Delta S)$ ($m\mu \cdot \text{cm}^{-1}$)
225	1	13.4	0.075
230	2	24.6	0.081
240	2	20.5	0.098
250	2	17.5	0.114
260	3	22.5	0.133
270	3	19.7	0.152
280	4	23.0	0.174
290	4	19.8	0.202
295	5	23.9	0.209

TABLE 2. SLOPE PARAMETERS AND C.V.(λE)

λ_m (m μ)	($\Delta S/\Delta\lambda$)	($\delta\log E/\delta S$)	($\delta\log E/\delta\lambda$)	c.v.(λE)
245	9.52	0.0193	0.183	0.292
270	6.58	0.0115	0.075	0.146
285	5.41	0.0032	0.017	0.088
290	5.00	0.0326	0.163	0.333
291	5.00	0.0396	0.198	0.515
293.5	4.83	0.0556	0.268	0.711
295	4.78	0.0696	0.332	0.725

with the relevant values of ($\delta\log E/\delta S$) and ($\delta\log E/\delta\lambda$). ($\delta\log E/\delta S$) was measured directly at each of the chosen wavelengths and ($\delta\log E/\delta\lambda$) calculated therefrom, using the appropriate values of ($\Delta\lambda/\Delta S$) interpolated from Fig. 2. To maintain a basis of comparison, the solutions of chlorocresol used to measure c.v.(λE) and ($\delta\log E/\delta S$) were of such concentration that $E(1\text{ cm})$ was close to 0.50 at λ_m , the wavelength of measurement. In this way, the contribution of photometric error was kept constant throughout.

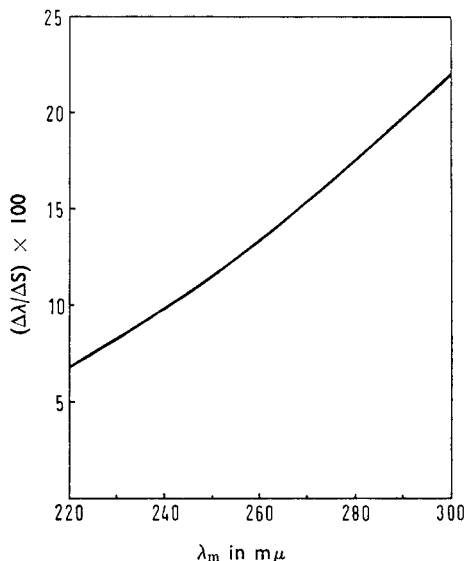


FIG. 2. Mean values of ($\Delta\lambda/\Delta S$) versus wavelength for the Uvispek photoelectric spectrophotometer.

Experimental

The measurements described in (a) and (c) below, employed solutions of chlorocresol (in 0.1N aqueous H_2SO_4) of such strength that $E(1\text{ cm})$ was close to 0.50 at all values of λ_m . In setting the wavelength scale to a given mark, the final movement was invariably made in the direction of higher wavelengths.

(a) DETERMINATION OF MEAN VALUES OF c.v.(λE). At a particular wavelength, λ_m , nine successive measurements were made of $E(1\text{ cm})$ of

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the appropriate solution, the first measurement being rejected on account of the considerable re-adjustment of the instrument controls, which normally preceded it. By re-setting the wavelength scale to λ_m before each measurement, the latter became subject to both wavelength setting and photometric errors. (No special device was used in setting the scale). The work was carried through in a series of blocks, each taking about 2 hr to complete and resulting in one estimate of $V(\lambda E)$, the variance of (λE) , for each of the seven wavelengths. Each value of the mean $c.v.(\lambda E)$ was then calculated from the mean $V(\lambda E)$ for the wavelength in question throughout the 29 blocks. In this way, fluctuations of operator and instrument performance contributed very little to the regression in Fig. 3.

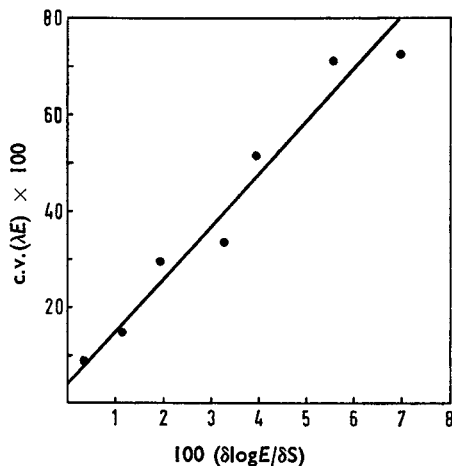


FIG. 3. Regression of mean $c.v.(\lambda E)$ upon $(\delta \log E / \delta S)$.

(b) MEASUREMENT OF $(\Delta \lambda / \Delta S)$ FOR THE UVISPEK PHOTOELECTRIC SPECTROPHOTOMETER. A magnified indication of the setting of the wavelength scale was obtained by means of the simple device shown in Fig. 1. It was free from backlash and easily adapted for measurements at any point on the wavelength scale. It consisted of a long thin duralumin pointer attached to one end of a small sheet of perspex, the opposite end of which bore a counterpoise. At its centre of gravity the whole assembly was mounted on a steel rod, and was kept in position by a setting knob and rubber washers. The steel rod was held in a rubber bung which could be tightly fitted into the hollow end of the spectrophotometer wavelength drum. In this way, the pointer was rigidly connected to the wavelength drum during measurements over a small interval of wavelength. It could be released by means of the setting knob, when the drum had to be rotated by more than a few degrees. Great care was taken not to strain the wavelength drum and so cause damage to the micrometer screw, which moves the prism table in the monochromator. For the same reason, the weight of the device was kept as low as possible. The pointer was 13 inches long and ended in a fine blade, which moved over a

scale attached to the front of the monochromator. The symbol, "S," refers to measurements along this scale. The latter was made by cutting out the scale of a thin plastic millimetre rule and forming it into an arc of radius 13 in. To obtain $(\Delta\lambda/\Delta S)$, the wavelength drum was set to the wavelength, λ_m , at which $(\Delta\lambda/\Delta S)$ was to be measured and the pointer clamped into position, so that it rested at about the middle of the scale. The position of the pointer was then noted after the drum had been accurately set to (i), $\lambda_m - (\Delta\lambda/2)$ and (ii) $\lambda_m + (\Delta\lambda/2)$, both wavelengths coinciding with scale graduations. ΔS was kept to the same order of magnitude throughout by suitable adjustment of $\Delta\lambda$. The data presented in Table 1 and Fig. 2 refer to means of 20 estimates of ΔS at each value of λ_m .

(c) MEASUREMENT OF $(\delta\log E/\delta S)$ FOR CHLOROCRESOL (IN 0.1N AQUEOUS H_2SO_4) AT VARIOUS λ_m . To determine $(\delta\log E/\delta S)$ at a particular wavelength, λ_m , the wavelength drum was set to λ_m and the pointer adjusted so that it rested at a point, S_0 , near the centre of the scale. Extinctions were then measured at 5 mm intervals along the scale, starting at $S_0 - 15$ mm and concluding at $S_0 + 15$ mm. In this way, a small section of the absorption curve (E against λ) was plotted out in the vicinity of λ_m and the slope, $(\delta E/\delta S)$, obtained therefrom. $(\delta\log E/\delta S)$ was then equal to $0.868(\delta E/\delta S)$ since $\delta\log E \approx 0.434\delta E/E$ and all solutions were of such concentration that $E(1\text{ cm})$ was close to 0.50 at λ_m . The same procedure was followed in triplicate for all values of λ_m quoted in Table 2. The readings which related to a particular λ_m were accumulated on one graph and the mean slope determined, the plots of E against λ being substantially linear in all cases. These measurements were of modest precision in view of: (i) small concentration differences between batches of solution; (ii) difficulty of reproducing λ_m in the initial setting of the pointer on the scale, and (iii) small drifts in the wavelength calibration, which occurred during the few days that were occupied with these measurements. For these reasons, the uncertainty in $(\delta\log E/\delta S)$ was probably of the same order as the uncertainty in the mean value of $c.v.(\lambda E)$. However, to have improved the precision of $(\delta\log E/\delta S)$ would have entailed an unjustifiable amount of additional labour, in view of the final objective.

Discussion

The use of $c.v.(\lambda E)$ instead of $\sigma(\lambda E)$ or $V(\lambda E)$ stems from the fact that for almost every analytical purpose, it is the *relative* extinction error, rather than the absolute error, that is important. This is most certainly true of Vierordt's method, which depends either implicitly or explicitly on the measurement of three extinction ratios (Glenn, 1960).

A graph of mean $c.v.(\lambda E)$ against $(\delta\log E/\delta S)$ is shown in Fig. 3 together with a regression line calculated by least squares. The value, 0.04, of $c.v.(\lambda E)$ at the intercept of this graph refers to $c.v.(E)$, the coefficient of variation of extinction measurements which are devoid of wavelength setting error, and is lower than actually experienced in this laboratory (0.07 for $E = 0.5$).

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The graph of mean c.v.(λE) against ($\delta \log E / \delta \lambda$) bore a close resemblance to that of Fig. 3, mainly because six of the seven wavelengths fell between 270 $m\mu$ and 295 $m\mu$, over which interval the change in ($\Delta S / \Delta \lambda$) is small. Nevertheless, an indication of what might have happened, if the wavelengths had been evenly spread throughout the range, 220 $m\mu$ to 300 $m\mu$, is given by the point for 245 $m\mu$. This was a notable outlier in the plot of mean c.v.(λE) against ($\delta \log E / \delta \lambda$) but not in Fig. 3, which supports the view that an operator's error in setting the wavelength scale to a given mark is independent of wavelength.

It is evident that c.v.(λE) bears an almost linear relationship to ($\delta \log E / \delta S$). Furthermore, relative to the usual value of c.v.(E), 0.07 for $E = 0.5$, c.v.(λE) is about ten times c.v.(E) for measurements on the steepest slopes and about five times c.v.(E) for measurements on average slopes—a statement, which refers to a particular combination of operator and instrument. Although a linear relationship between c.v.(λE) and ($\delta \log E / \delta S$) probably applies to all combinations, the regression constant may be subject to variation.

The steep linear slope of the relationship between c.v.(λE) and ($\delta \log E / \delta S$) is of no small relevance to the choice of wavelengths for Vierordt's method, for it is obviously desirable to avoid very steep slopes, even at the cost of an increase in purely photometric error. By ignoring the important matter of slope, one of the methods suggested by Stearns (1950) is not altogether satisfactory since a graph of

$$[(E 1\%, 1 \text{ cm}) \text{ of A}] / [(E 1\%, 1 \text{ cm}) \text{ of B}]$$

against wavelength obscures the slopes of the absorption curves which contribute thereto. The second method recommended by Stearns and described in greater detail by Glenn (1960), involves a super-position of the $\log E$ vs. wavelength curves for the two components and so gives a clear indication of the slopes which will be encountered at any given wavelength. Furthermore, this second method usually involves less labour than the first.

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