SECTION OF ANALYTICAL CHEMISTRY

COMMISSION ON OPTICAL DATA*

REPORT ON CALIBRATION OF WAVELENGTH AND PHOTOMETRIC SCALES OF NON-RECORDING SPECTROPHOTOMETERS

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1. INTRODUCTION

The spectrophotometer is an instrument which is used to measure the intensity of a beam of light for a narrow range of wavelengths. There are, therefore, two principal quantities to be determined:

(a) the region of wavelengths, which is a dispersion problem, and

(b) the intensity of the beam, which is a photometric problem.

These two are connected. There may be errors in the measurement of both quantities. There may be an error in the reading of the wavelength scale, and, as a result of this, the intensity which is measured at the spurious wavelength is incorrect. The actual measurement of intensity may be subject to an error, owing to the photometric means employed. It follows that the permissible error in wavelength reading is that which causes an error in intensity determination not greater than that which the photometric measurement entails. Thus the two quantities are connected through their respective errors, which should be matched. Nevertheless, it is permissible and convenient to consider them separately, and this will now be done, after defining the terms which are used.

Because of their widespread use, the spectrophotometers considered in this report will, unless otherwise stated, be of the photoelectric non-recording type. The report envisages the employment of such instruments as being the normal one of measuring absorption only. The purpose may be:

(a) analysis of samples, by measurement of their absorption (the purpose with which this report is mainly concerned),

(b) determination of structure of compounds,

(c) measurement of the intensity of the radiation transmitted.

These may be further broken down; for example, under (a) one may wish to measure the absorption of a sample over a wide range of wavelengths, or may wish to determine very accurately the absorption in a few wavelength regions. Further, one may wish to measure a small change in absorption in one particular spectral region, due to changes in other parameters such as temperature, dilution and so on. According to the nature of the problem, the error which can be tolerated in wavelength or intensity reading varies in magnitude.

2. NOMENCLATURE

2.1—In a preliminary version of this report, attention was drawn to the chaotic state of the nomenclature of absorption spectrophotometry, and the writer suggested that the Commission should consider this and make recommendations. This was done at a meeting in Liège in 1958. Since the agreed terminology is used in this report, the conclusions are here reported. (In the meantime, this question was also examined by the Commission on Molecular Structure and Spectroscopy and its decisions were reported in *Information Bulletin* No. 8. An examination of both sets of decisions shows them to be in practically complete agreement, the only difference being that some extra terms were agreed by the Commission on Optical Data. To distinguish them, they are put in square brackets in the following list.)

2.1.1—The unit of wavelength is the ångström $(Å)$; the millimicron $(m\mu)$ is usable when appropriate to the coarseness of the instrument being used. [The term nanometer (nm) is recognized as synonymous with millimicron, and is helpful when a typewriter is used.]

2.1.2—The symbol of wavelength is λ.

2.1.3—Concentrations (c) are expressed in moles/litre.

2.1.4—Sample thickness (b) is expressed in centimetres. The symbol l is not liked, since it may easily be confused with the digit 1.

2.1.5—Incident and emergent intensities are written as I_0 and I respectively. $[\Phi_0$ and Φ for these quantities are tolerated.]

2.1.6—The extinction coefficient, ϵ_λ, is such that

$$\epsilon_\lambda = \frac{\log_{10}(I_0/I)_\lambda}{cb}$$

the units being $(cm\ mole/litre)^{-1}$.

2.1.7—[The quantity $\log_{10}(I_0/I)$ is known as the absorbance, A. The expression optical density and the symbol D are also recognized.]

2.2—To make clearer the meaning of these terms, consider the measurement of the absorption of a solution placed in a cell in the dispersed beam issuing from a monochromator. Suppose that immediately after entering the liquid the beam has intensity I_0, and contains wavelengths over a range $\delta\lambda$, the mean wavelength being λ. The beam traverses a path of

length b in the solution, and the intensity of the beam is then I. It will have decreased exponentially because of the absorption, so that

$$I = I_0 \times 10^{-\epsilon bc}$$

Hence the form given in **2.1.6**. It is important to consider the incident beam immediately after first entering the solution, so as to avoid complicating the definitions by considerations of the reflection losses at the walls of the cell, and absorption in the cell wall.

3. THE WAVELENGTH SCALE PROBLEM

3.1—If the sample has a small variation in absorption with wavelength, the wavelength reading need not be precise, but if a material such as a didymium salt is the sample a small error in wavelength setting may have a large effect on the apparent absorption.

3.2—Testing the scale

The simplest way to test the wavelength scale in the visible region is to use a line spectrum, such as is provided by a mercury lamp. With a narrow entrance slit, reduced to the stage when further reductions in width reduce brightness without reducing the apparent width of the line seen at the exit slit, and with the exit slit set so as just to embrace the spectral line, the wavelength indication should be that of the mercury line being examined. If now the slits (assumed symmetrical) are widened to the extent customary in the use of the instrument, the reading should remain the same, unless the prism is not being used at minimum deviation. With a wide entrance slit and the prism off minimum deviation, rays from one edge of the slit fall on the prism in a direction nearer to that of minimum deviation than rays from the other edge. Consequently, the deviation of the rays is not symmetrical about the mid-position, so that the mean wavelength changes as the slit is opened, when one considers a continuous source. Fortunately, most of the spectrophotometers under consideration use a Littrow system, in which the line under examination is produced by rays which pass through the prism close to the direction of minimum deviation. For radiation in the ultra-violet region, similar observations can be made when a screen of fluorescent material, such as uranium glass, is placed over the slit.

Wavelengths suitable for the purpose are given in *Table 1*, at the suggestion of M. Loeuille of Paris, who supplied the author with a list. This list has been modified and extended, and the suitability of the lines included has been specially checked in the author's laboratory. Some lines which might have been expected to be included have been omitted, either because they are susceptible to self-reversal (for instance, Hg 2537), or because other strong lines are so close that in an instrument with low dispersion the wavelength would be in doubt. The wavelengths (with the exception of the first line), are taken from Harrison's tables, and are given in ångströms, to the first decimal figure. They should be rounded off to the degree warranted by the precision of the instrument used (four significant figures for most instruments, three for the coarse ones). The fifth figure has been included so as to provide for greater resolution possible in the future. It

is not necessary to have all the sources listed, unless a very detailed calibration is required; for most workers a (low-pressure) mercury lamp will suffice. A neon lamp is useful if the visible range is especially important.

Table 1. Useful spectral lines for wavelength calibration (in Å)

Mercury	Neon	Helium	Rubidium	Zinc	Cadmium
1942·3	5330·8	3888·6	4201·9	2025·5	2677·6
2302·1	5341·1	4471·5	4215·6	2061·9	3261·1
2378·3	5400·6	4921·9	5578·8	3282·3	3403·7
2446·9	5852·5		7800·2		3610·5
2752·8	5944·8	*Sodium*	7947·6	4680·1	4678·2
2803·5	6143·1			4722·2	4799·9
2893·6	6334·4	5682·7	*Caesium*	4810·5	5085·8
3125·7	6402·2	5688·2		6362·3	6438·5
3906·4	6678·3	5890·0	4555·4		7346·2
4046·6	6929·5	5895·9	4593·2	*Hydrogen*	
4077·8	7173·9		8521·1		*Thallium*
4358·4	7245·2	*Potassium*	8943·5	4101·7	
5460·7				4340·5	2767·9
5769·6	*Krypton*	4044·1	*Argon*	4861·3	2918·3
5790·7		4047·2			3229·8
	4274·0	7664·9	8115·3		3519·2
	8928·7	7699·0			3775·7
					5350·5

3.3—Sometimes the exit slit is opened wide, and a transparent plate carrying cross-wires is placed over it with the mid-point of the cross-wires half-way between the jaws. The entrance slit is made narrow and the spectral line is moved into coincidence with the cross-wires. For the ultra-violet region, a uranium glass plate with cross-wires is used. Such a method requires the exit and entrance slits to be independently adjusted.

3.4—An alternative procedure uses the method of paragraph **3.2**, substituting the electrical measuring system for the eye, and seeking for a maximum emission. This method is to be preferred to the foregoing ones, since with prism instruments the spectral line is curved, so that the electrically effective position of the line with regard to the exit slit is not quite the same as that of the middle of the line. In principle the best method is likely to be that one which most nearly follows the procedure necessary in using the instrument in the normal way. According to this principle this electrical method is to be preferred.

3.5—Interference method

A different approach is to use Edser–Butler fringes[1] to produce maxima and minima in the spectrum of a continuous source. This has been used by Rank, Rix and Wiggins[2] for calibration of absorption spectra and applied to the calibration of spectrophotometers by Heidt and Bosley[3]. Rank *et al.* used bare glass plates, to which the simple theory applies, *viz.* $P\lambda = zt$, where P is the order of interference and t is the geometrical separation of the plates. Heidt and Bosley used aluminized surfaces to increase the

reflectivity, but do not appear to have made the correction for the phase change on reflection at the metal surfaces. This may make a difference of several ångströms when carrying over to 2000 Å an effective gap separation determined at 3500 Å, for gaps as small as those actually used (of the order of 10^{-3} cm). Heidt and Bosley obtained absorbance wave curves by placing a narrow-gap étalon in the sample compartment of the spectrophotometer, and standardized the gap by superposing the record of the hydrogen lines occurring in the lamp used for the ultra-violet. From this, estimates may be made of the positions of the maxima and minima, and these were used to check the wavelength scales. If found necessary, a calibration curve can be constructed.

Buc and Stearns[4] used an analogous device which produced interference fringes by polarization with a retarding plate. If a medium other than air is used, the dispersion of the medium must be taken into account.

4. EFFECTS DUE TO FINITE WIDTH OF SLITS

As customarily used, the slits of the spectrophotometer may have to be wide, so that sufficient energy reaches the detector. Used in conjunction with a source of continuous radiation, the exit slit will pass light having a range of wavelengths. The range will be greater, the wider the slits and the less the dispersion of the instrument. It may also be impure, in the sense that radiation of very different wavelengths is present owing to stray light—this is considered in paragraph 5 below.

4.1—Width of band of wavelengths passed. (Diffraction ignored)

Suppose F_1 and F_2 are the focal lengths of the collimator and " camera " lenses respectively. Imagine a finite entrance slit, of width W_1 and a narrow exit slit. If D is the angular dispersion $(d\theta/d\lambda)$, $W_1/F_1 = D\delta\lambda$, so that the band of wavelengths emerging through the narrow exit slit is given by

$$\delta\lambda = W_1/F_1 D$$

Suppose now that the exit slit is widened to W_2. Considering rays from the extreme edges of W_1, it will be seen that the band now emerging from the exit slit is of width $\Delta\lambda$ given by

$$\Delta\lambda = W_1/F_1 D + W_2/F_2 D$$

As normally used, the width W_2 of the exit slit is matched to the width W_1 of the entrance slit, so that $W_1/F_1 = W_2/F_2$ and

$$\Delta\lambda = 2W_1/F_1 D = 2W_2/F_2 D$$

$\Delta\lambda$ here is the complete range of wavelengths passed, without reference to the flux associated with the different wavelengths.

4.2—Effect of diffraction

If a narrow entrance-slit is illuminated by a monochromatic source of wavelength λ, there will be a widening of the line-image due to diffraction.

If B is the breadth of the beam of light emerging from the prism, then the (half)-width W_d of the flux wavelength plot is given by

$$W_d = \lambda F_2/B$$

if a square cross-section is assumed for this beam. If the entrance slit is now opened, so that the *image* of this in the plane of the exit slit is W_2, Kaiser and Hansen[5] have shown that the effective slit width W_{eff} is given sufficiently nearly by

$$W_{\text{eff}} = W_2(1 + W_d^2/W_2^2)^{\frac{1}{2}}$$

Thus, when the entrance slit is narrow, the effective width is mainly due to diffraction, while when the entrance slit is wide, the effect of diffraction becomes negligible. Applying these considerations to **4.1**, one arrives at

$$\Delta\lambda = W_{\text{eff}}/DF_2$$

where $\Delta\lambda$ is the width of the waveband at half-intensity.

4.3—Variation of flux passed with wavelength

So far, only the wavelengths passed have been considered, without reference to the flux associated with each wavelength. Naturally this will depend upon the variation of flux with wavelength in the source, and various attempts have been made to consider the general case. For example a general treatment has been given by Hardy and Young[6], and Eberhardt[7] has considered the case of matching entrance and exit slits, with examples of application to particular absorption bands. For a detailed treatment of the waveband problem, see the paper by Perry[8].

For the purpose of this report, the ideal case may be considered of a waveband over which the flux associated with each wavelength is constant, and the dispersion is normal over the waveband. If diffraction is ignored, and the slits are matched, the extreme wavelengths will be associated with zero flux, while for the central wavelength the flux will be a maximum; the flux–wavelength plot is triangular.

If the exit slit is wider or narrower than the image of the entrance slit, the plot is trapezoidal; the flat top is of width $|(\delta\lambda)_2 - (\delta\lambda)_1|$, the base of width $(\delta\lambda)_2 + (\delta\lambda)_1$, where $(\delta\lambda)_2$ is the waveband corresponding to the actual exit slit, and $(\delta\lambda)_1$ is that covered by the image of the entrance slit. The half-flux width is seen to be either $(\delta\lambda)_2$ or $(\delta\lambda)_1$, whichever is the greater.

For the normal purposes of analysis with the spectrophotometers here considered, a triangular plot is sufficient.

It is to be noted that for matching slits the half-flux width, expressed in wavelengths, is the same as the total waveband passed when the exit slit is very narrow (ignoring diffraction).

For non-matching slits, starting from the condition of match, as the exit slit alone is progressively widened, the flux associated with particular wavelengths under the flat top is unaltered, but the total flux increases, as does also the width of the waveband. If from the state of matching,

the exit slit is progressively decreased, the total flux is reduced, without gain in purity. Consequently to match the slits is advantageous, unless the energy is insufficient.

5. STRAY LIGHT

5.1—In well designed spectrophotometers this should be negligibly small over the useful range of the instrument, if it is in good condition. If it is not well designed, or if the optics have been allowed to become dusty, light will be scattered in unwanted directions and some may emerge from the exit slit mixed with the wanted radiation. With continuous illumination, the ratio of stray light to wanted light is independent of slit width, but with monochromatic illumination, it is proportional to the slit width.

The effect of stray light is to give spuriously high or low transmittance, according as the absorbing substance has a higher or lower transmission for stray light than it has for the wanted light. Suppose T is the true transmittance of the sample for the wanted wavelength, and T_s is the true transmittance of the sample for the wavelength of the stray light. Let T_{app} be the apparent transmittance of the sample as measured in the presence of stray light. Let I_0 be the intensity of the incident beam of wanted wavelength, and S_0 be the intensity of the incident stray light.

Then
$$T_{app} = (I_0 T + S_0 T_s)/(I_0 + S_0)$$

or
$$T_{app} - T = S_0(T_s - T)/(I_0 + S_0)$$

So if $T_s > T$, $T_{app} > T$ and conversely.

Consequently if T is known, measurement of T_{app} with the spectrophotometer shows whether it is equal to T or greater or less. A special case could arise when the stray light had wavelengths belonging to two (or more) regions of the spectrum, of such a kind that apparently the true transmittance was measured, falsely. This, if suspected, could be shown up by measurements of different samples having absorption bands in different places.

Stray light is normally lower in systems employing double monochromators, and with single monochromators the right use of auxiliary filters may help in a similar way, by diminishing the intensity of unwanted wavelengths.

It is convenient to distinguish between two types of stray light, *viz.* that due to wavelengths far from the wavelength region wanted (" far " stray light) and that due to wavelengths close to the waveband being measured.

5.2—" Far " stray light

The simplest method of *detecting* such stray light, is to measure the apparent density of an absorbing substance in the wavelength region being investigated, and then to introduce into the path of the incident radiation an optical filter which transmits the region required, but obscures other regions. If the density now measured is different, some scattered radiation has been removed.

5.3—An estimate of the amount of stray light is sometimes made by using the formula of paragraph **5.1**, and putting T_s arbitrarily equal to unity, so that

$$T_{app} - T = S_0(1 - T)/(I_0 + S_0)$$

or $\qquad S_0/I_0 = (T_{app} - T)/(1 - T_{app})$

A measurement of T_{app} is made of a substance for which T is known. Clearly this is no more than a rough estimate, for there is no justification for regarding T_s as unity. Unless the wavelength region of the stray light is known, a *measurement* cannot be made of the stray light in this way. A satisfactory method is given in paragraph **5.5** below. If a good double monochromator free from scattered light is available, light from this can be fed into the spectrophotometer under test, and the output can then be measured for different settings of the wavelength of the instrument under test. This has been done by Pritchard[9].

5.4—Another method of estimating stray light, described by Hogness, Zscheile and Sidwell[10], which does not require a substance of known density, can be applied when it is possible to increase the length of path through an absorbing liquid, or to increase the concentration. (The former is preferable, since the latter may cause a change in absorption due to factors affecting the validity of Beer's law.) If stray light is absent, the density will increase progressively, but if it is present, the density will rise to a maximum value, as $d = \log_{10}(I_0 + S_0)/(I + S)$ tends to $\log_{10}(I_0 + S_0)/S$.

5.5—" Near " stray light

Stray light due to wavelengths near to those of the waveband being studied may be very troublesome, and it will not readily be detected by the foregoing methods. Preston[11] measured stray radiation by a technique which can measure both kinds of stray light. He assumed that the stray radiation illuminates both halves of the slit equally. The method is to fix a plate rigidly on the entrance slit so as to cover a little more than half its length. A similar plate is arranged to block *exactly* half the exit slit, the half being blocked corresponding to the image of the open half of the entrance slit. This plate may be put in position or removed at will. When the first screen only is in place, the wanted beam emerges from just less than half the exit slit, while scattered light fills the exit slit symmetrically. When the second plate is also in position, the wanted beam is eliminated and half the stray light emerges. The application of the method may be shown by considering the measurement of the true transmission of a filter (free of error due to stray light).

Four readings are taken, all with the plate on the entrance slit:

R_1	No screen on exit slit	No filter
R_2	No screen on exit slit	Filter in place
R_3	Screen on exit slit	Filter in place
R_4	Screen on exit slit	No filter

The apparent transmission of the filter will be R_2/R_1. The true transmission will be $(R_2 - 2R_3)/(R_1 - 2R_4)$. If these two are equal, there is no stray light. This method is unduly long for use as a routine, but it is recommended for detecting and measuring stray light if it is suspected. For this test, in the form presented by Preston, it is necessary that the exit and entrance slits should be separate.

Tunnicliff[12] has described a method of measuring near stray light, based on the use of the narrow absorption band in mercury vapour at 2537 Å. This becomes broader as the temperature of the vapour is raised.

Stray light has been examined theoretically by Perry[13] and some practical examples are considered by Holiday and Beaven[14].

6. THE INTENSITY SCALE PROBLEM

6.1—The photoelectric spectrophotometer is provided with a scale engraved in terms of optical density, transmittance, or both. It is required to know what confidence can be placed in the graduation, and if necessary what curve should be constructed to calibrate the readings. (The latter procedure is not recommended; it is better to change the offending component, probably photocell or potentiometer.) These requirements are met to a different extent by the following three types of method in general use, which may be distinguished as:

(a) *Physical absorption methods*, which employ diaphragms, polarizing arrangements and the like, simulating absorption of calculable amounts. Since the absorption is calculable, these are absolute methods.

(b) *Chemical absorption methods*, which employ absorption by solutions having " known " optical densities. Since one cannot calculate the optical densities, one must measure them on an already calibrated instrument. These are, therefore, comparative methods, which, however, are very convenient.

(c) *Electrical methods*—These are not absolute methods, in that, for example the linearity of the photocell must be assumed to be satisfactory, or be determined separately. They are extremely convenient, and if the instrument satisfactorily passes these tests it is almost certainly correct, for an unusual combination of circumstances is required to render them nugatory.

7. PHYSICAL ABSORPTION METHODS

7.1—Variable aperture

This method is extremely simple, but requires great caution in its application.

An aperture is easily made variable, and is placed in a beam which is made as uniform as possible over the whole cross-section of the aperture opened to the greatest area used. If the beam is uniform, then the flux passed will be directly proportional to the area of the opening. The image of the opening, when enlarged to the maximum used, must fall within the aperture stop of the instrument—otherwise at some point when it is being

opened, the relationship between flux and area will fail. Good homogeneity of the beam is easier to attain if the lens or mirror used to form the beam is used at a low relative aperture. Examples of the use of variable apertures are the Spekker photometer of Hilger and the Pulfrich photometer and Elko II of Zeiss.

7.2—Grids

The grids or gratings are formed for example from wire mesh, and allow light from different zones of the lens forming the beam to pass simultaneously. Each such grid has a fixed transmittance, and the grids are interchangeable. Unfortunately, owing to diffraction effects, grids are not neutral, having a different transmission for long waves than for short. They are convenient and do not easily suffer from ageing effects. The beam need not be as homogeneous as when the gross aperture type of attenuator is used.

Grids have been revived recently by Heidt and Bosley[3] who used screens of single strands of brass wire, made sufficiently small to be placed in the sample tray in the spectrophotometers they investigated. They used screens singly and in tandem, combining up to six screens, and naturally found that the relative orientation of screens in tandem had a marked influence. They found the density to be dependent on wavelength, and expressed this dependence by a formula. While this detracts in principle from the usefulness of such screens, they stated that the screens could be treated as neutral " in the first approximation ", by which they mean that " the uncertainties in the absorbance values in any case are no greater than those encountered in the use of potassium chromate " (see below).

(The calculated value of the percentage open area should be $(1 - ms)^2$, where m = mesh in number of wires per inch, and s = diameter of the " wire in inches ", and not as printed in the paper.)

A grid of special design due to Kaiser[15], which has several advantages over fixed screens, may be mentioned here. A plate is pierced with holes which are made with a precision drill, and the holes are opened out on each side of the plate so that they have the precise diameter half-way through the plate, but the thickness of the plate does not obstruct the angular aperture of each hole. The transmission may be calculated from the number and diameter of the holes. The plate has an oval rim, resting on two rollers; as the rollers rotate the plate " tumbles " in the beam, randomizing the positions of the holes.

7.3—Rotating sectors

7.3.1—*Simple type*—This is a rotating " chopper ", which consists of an opaque disc with a sector missing, so that in each period the light passes for a time depending upon the angle of the sector. The sector may be made to be adjustable, by superposing on the same axle two discs each with two 90° sectors missing, and adjusting one with respect to the other so that effective sector openings from 90° to zero may be obtained. For this purpose it is customary to mark a degree scale (or a percentage scale) on one of the opaque sectors. Since there are two openings, the transmission varies from 50 per cent to zero. It is also possible to have curved

sectors, so that for example a logarithmic law may be followed. Such an attenuator operates not by reducing the intensity of the light, but by reducing the time during which it operates. Consequently errors will result if the period of flicker is not small compared with the time constant of the measuring circuit. Thus in practice such a device may only be used with an integrating device, or one having a long time constant. Success has been achieved with rotating sectors in photographic photometry, but this simple type is not suited to photoelectric spectrophotometers.

7.3.2—*Hollow axle rotating sector*—Pool[16] devised a special form of rotating sector which may be used with photoelectric spectrophotometers, to reduce the intensity in a calculable way. It reduces the intermittent effect in practice to a small amount—with perfect optics and adjustment it would be zero. The sector is mounted on a hollow axle supported by a ball-race round it, and it is driven by a belt, so that the whole beam passing through enters the spectrophotometer. The image of the sector is arranged to fall within the stop of the spectrophotometer. The writer introduced some modifications to Pool's form of the apparatus. In this a " lid " is placed on the rotating hollow cylinder. The lid is formed from a ring of stout metal which fits over the end of the hollow cylinder, with a sheet of metal screwed onto the ring. Consider an attenuation of one half. The semi-circular sheet of metal which forms the obscuring half needs only to have the edge straight which constitutes the diameter of the opening. This is adjusted by means of the screws, so that when the sector rotates, neither a black centre nor a white centre on a grey ground can be seen. It is very easy to secure this exact adjustment.

If the beam were completely homogeneous, then exactly half the intensity would be obtained (this would also be true if the sector were at rest). The effect of the rotation is to use different portions of the beam in turn. If the beam is not truly homogeneous, then there is an intermittent effect, but only in proportion to the degree of inhomogeneity of the beam. If the sector rotates rapidly enough, the intermittent effect is not harmful. With a photoelectric spectrophotometer there was no flicker of the meter needle to be seen. Openings of other sizes can be adjusted in a similar manner, but already much can be done with a half-intensity device, since one may arrange for any setting of the intensity scale to correspond to full opening of the sector, and then to read the half-value.

7.4—Polarization devices

With a pair of nicol prisms in series in the beam, one may reduce the intensity of the emergent beam in a calculable way by orienting the principal plane of one with an angle ϕ relative to that of the other, since the transmittance is proportional to $\cos^2\phi$. Such a device can only be used over the range of wavelengths transmitted, which in practice means the visible and near ultra-violet regions. (Polaroid is unsuitable because of the partial transmission in the violet and red of crossed polaroids.) The small aperture of the nicol also introduces a difficulty. If the density required is high, ϕ must be large, and since $dI/I = 2 \tan \phi \, d\phi$, a small error in adjusting ϕ leads to a very large error in intensity.

7.5—Neutral filters

If there were a strictly neutral filter, it would be possible to construct wedges of the material which could be used to calibrate the intensity scale. Actually no truly grey material has yet been made. For some purposes finely dispersed particles of such substances as carbon, or finely divided metals embedded in gelatine or glass, have been used. Partly exposed and developed photographic plates prepared with finely divided silver constitute such a filter in effect. They are not truly neutral, and have to be standardized in some way so that, though convenient in use, they are secondary standards, and should be calibrated for different wavelength regions. A good glass filter of this type serves as a convenient check to apply from time to time.

The interposition of a glass filter may introduce trouble due to light losses by reflection at the surfaces of the filter. Cary has greatly reduced this effect, by having a trough of liquid in place all the time, and introducing the filter by plunging it into the liquid (*e.g.*, oil). The liquid is chosen so as to have a refractive index sufficiently near that of the glass to give rise to little reflection at the liquid–glass interfaces.

7.6—Inverse square law of distance

Use may be made of this law for the satisfactory calibration of a filter to be used with a spectrophotometer, in the visible or ultra-violet regions, if certain precautions are taken. Kaiser[15] has described such a method, and the errors to be avoided. The lamp is placed in a light-tight housing, with a sharp-edged window. The light passes through suitably arranged windows and falls on a flat surface, coated with magnesium oxide. The distance between this surface and the lamp may be varied, so as to vary the intensity of illumination of the surface in a calculable way. The light must not be allowed to fall directly on the receiver, first because the cathode of a photocell or photomultiplier is insufficiently flat, and secondly because trouble may be caused by shading, *e.g.*, by anode wires. This may be avoided by introducing an integrating sphere (see also Grossmann, Sawyer and Vincent[17]).

8. CHEMICAL ABSORPTION METHODS

These employ solutions of definite composition, concentration and thickness, for which the optical density is believed to be known. The absorbing material must be easily obtained in a pure state, and have absorption bands which are flat-topped—*i.e.*, have maxima (or minima) of absorption which change only slowly with change in wavelength (otherwise the error in photometry may be affected by error in the wavelength scale). If an error in the wavelength scale is suspected, the measurement of density may be made at the maximum (or minimum) density. A good example of the use of this method is the comparative tests carried out by the British " Photoelectric Spectrometry Group "[18, 19], which stimulated the setting up afterwards of similar tests by the Dutch " Werkgroep Voor Photoelectricshe Spectrophotometrie "[20]. These show that with normal care, in most chemical laboratories a reproducibility of about 1·5 per cent

may be achieved. With considerable care, especially giving attention to cleanliness, and measuring the absorption at an agreed temperature of the liquid, it should be possible greatly to reduce this (see, for example, the work of Haupt[21]). Collaborative tests in America have been reported by Brode, Gould, Whitney and Wyman[22], by Vandenbelt[23] and by Vandenbelt and Spurlock[24]. A discussion of collaborative tests has been published by Cahn[25], and Gridgeman[26] has given a general discussion of the reliability of spectrophotometry.

9. ELECTRICAL METHODS

9.1—In the *making* of photoelectric spectrophotometers, the simplest way of ensuring an optical density scale accurate within the tolerance designed, is to select photocells which are sufficiently linear in the light-intensity–current characteristic (fortunately, this is easy), and to select potentiometers and fixed resistors having resistances within the tolerances calculated as being necessary to obtain the overall accuracy. (No compensatory design is practical because of the possibility of spares being required.) The linearity of the photocell guarantees that potentials developed across a fixed resistor will be proportional to the light fluxes received, and the linearity of the measuring potentiometer ensures that potentials will be accurately determined by the reading of the scale attached to the potentiometer. The spectrophotometer of the type being considered is a potential-comparing device, using a resistance bridge. When the potentiometers are checked for errors, the errors found in rejected potentiometers are random. It is likely, therefore, that the photocells in the instrument are sufficiently linear, and that if the potentiometer is faulty the errors are random.

9.2—Single filter method

This is a very simple test to apply, and if the instrument fails on this test there is certainly something wrong. If it does not fail on this test, then the instrument is proved reliable, *unless* (as pointed out by Cannon[27]), the photocell has a current characteristic proportional to a power of the light-intensity $(i \propto I^n)$. So far, in the author's experience, this has not occurred within the current range used in these spectrophotometers so that in practice the test may be relied upon. If there should be doubt on the linearity of the photocell, measurement of the optical density of a single standardized filter should dispel it, since the optical density will be measured as nd for the law just considered and the correct results will only be obtained if $n = 1$.

With the scale reading 100 per cent transmission, and no filter in the way, the meter needle is balanced with the 100 per cent adjustment. The filter is now inserted, and the transmission (say x per cent) determined in the ordinary way. This is now repeated, with the photometer scale at some other reading, *e.g.*, m per cent, at the commencement, and the needle is balanced. The filter is inserted again, and a new transmission determined, which should read $mx/100$ per cent if the scale is correctly calibrated. This can be repeated for various values of m. A good procedure is to use as a

filter a trough of absorbing solution, with a trough containing solvent only for comparison. This follows closely the normal use of the instrument.

The simplest procedure is to work with density readings, naturally commencing with the normal use of the 100 per cent setting knob (" check " knob) to obtain density zero. Insertion of the solution gives a reading of a. Now the check knob is set in such a position that with the solvent trough in place, balance is obtained for a reading a. Replacement of the solvent by the solution will now yield a new density reading, b. The process is repeated, now starting with b. If the instrument is properly calibrated, the readings a, b, c, etc., should form a series in arithmetical progression.

As an example the figures in *Table 2* are given. They were obtained by the writer in checking an instrument, with potassium dichromate solution against water, for wavelength 313 mμ.

Table 2. Scale readings

With water	With solution	Calculated series
0·000	0·124	0·1243
0·124	0·249	0·2486
0·249	0·373	0·3729
0·373	0·496	0·4972
0·496	0·621	0·6215
0·621	0·745	0·7458
0·745	0·870	0·8701

The figures in the first two columns will be seen to be in arithmetical progression, being approximately multiples of 0·1234. Incidentally, the biggest error in this test where the reading was 0·496, corresponded to an error in transmittance of one-quarter per cent.

9.3—Two-filter method

A variant subject to the same proviso as in paragraph **9.2** is to use two filters, measure the density of each separately, and then measure the density of the two combined. Since this should be the sum of the two densities measured separately, the calibration of the scale can be checked. The procedure may be repeated as before, with intial balancing at different positions on the scale, or by keeping the initial setting as 100 per cent transmission, and using different filters.

9.4—Added light method

Hansen[28] describes a difference method which uses a similar principle, namely that the response to two sources simultaneously should be the sum of the responses to the two sources separately. The special feature is that he adds a constant small light-flux from a second lamp (of constant output) after each reading, and reads the new deflection of the meter. If the instrument is correctly calibrated, the increment of reading should be the same. This method is not subject to the proviso of paragraph **9.2**, i.e., the response of the detector may be any function of the incident light-flux.

10. PREFERRED METHODS

When several good methods of test are available, the writer prefers that which most nearly follows the natural procedure in using the instrument. For testing wavelength scale calibration, therefore, he prefers the method of paragraph **3.4**, and for testing photometric scale calibration one of the methods of paragraph **9**.

11. NOTE ON CLEANLINESS

Though not strictly within the scope of this report, it is not possible to lay enough stress on the need for scrupulous cleanliness if consistent results are to be obtained. Professor Pestemer of Leverkusen keeps his cells when not in use in a liquid, so that the inner cell walls are never dry, and the cells can be flushed out before use.

12. NOTE ON EFFECT OF TEMPERATURE

It is not always realised that the temperature coefficient of absorption may be high enough to falsify the observations, particularly when accurate results are needed. This may account for some part of the discrepances observed in collaborative tests (see paragraph 8). Haupt[21] (*loc. cit.*, p. 445) measured the transmission of an alkaline solution of potassium chromate over a range of temperature of 25°C, and gives detailed observations. The results appear to indicate that the absorption bands move towards longer wavelengths with increase in temperature.

13. NOTE ON ABSORPTION CELLS

Each cell must have parallel walls, and pairs of matched cells should be truly matched. A test of this matching can be made by making an observation in the usual manner, and then a further observation after interchanging the contents of the respective cells. Care should be taken that the cells are placed with their walls perpendicular to the light beam. If the normal to the cell walls makes an angle θ with the light beam, and the thickness of the cell is b, then the path through the cell is now $b' = b \sec \theta / \mu$, where μ is the refractive index of the contents. This makes the fractional error in path $(b' - b)/b$ equal to $\theta^2/2\mu^2$, and this is the same as the fractional error in optical density which can be tolerated. For aqueous solutions, and an error of one part in a thousand, this necessitates an accuracy of setting of about 3°, which is easy to achieve.

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