Nov. 17, 1959 R. H. HAMILTON 2,912,895

SPECTROPHOTOMETRY PROCESS

Filed March 18, 1954

FIG. 1.

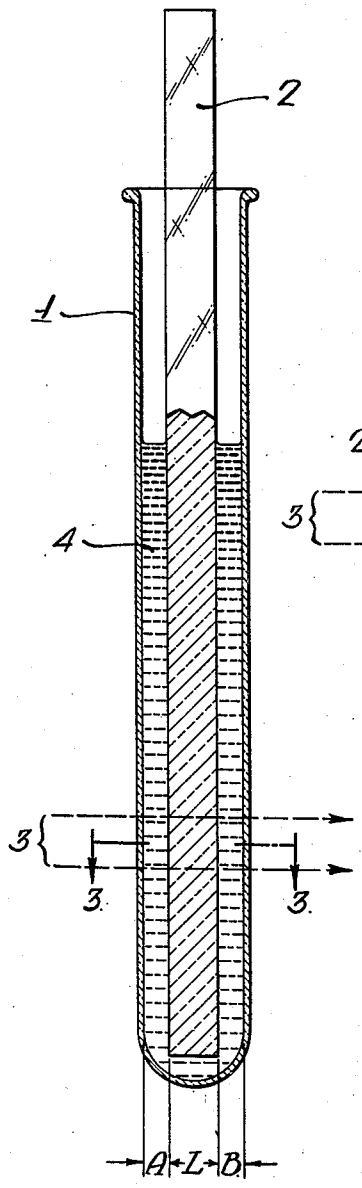


FIG. 3.

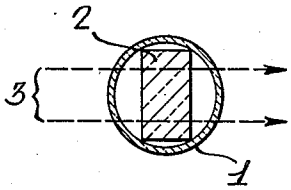
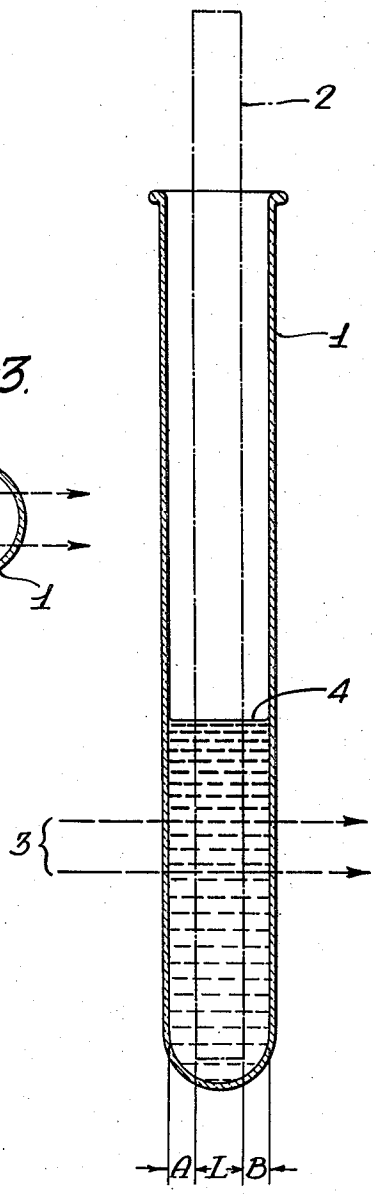


FIG. 2.



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SPECTROPHOTOMETRY PROCESS

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1 Claim. (Cl. 88—14)

This invention relates to improvements in spectrophotometry, and a principal object of the invention is to provide an improved process of displacement or absorption spectrophotometry as hereinafter set forth.

One of the problems encountered in laboratories using test tube type absorption cells for spectrophotometry is the securing and maintenance of sets of matched tubes of sufficiently close characteristics. To circumvent this difficulty, displacement plates can be used to allow displacement and replacement of layers of the colored fluid of uniform thickness. Critical study of the use of such plates shows a high degree of accuracy to be attainable. Results further indicate the possibility of eliminating need for transferring photometric solutions from the "working" test tubes in which previous analytical steps were carried out to an "optical" set of test tubes in which photometric readings are made. Limitations of the method are discussed, and possibilities are considered for selection of suitable displacement plates.

The invention will be more readily understood by reference to the attached drawings, wherein: Fig. 1 is a sectional elevational view showing apparatus capable of use in the practice of my invention, and illustrating an initial step of the operation; Fig. 2 is a corresponding view illustrating a subsequent step; and Fig. 3 is a transverse sectional view on the line 3—3, Fig. 1.

In the usual absorption photometry of solutions, light of restricted wave length incident on the photocell or phototube is set to read unit intensity after passing through a cell containing solvent or solvent plus the amount of impurities in reagents (blank). Then, light intensity being maintained constant, an identical cell is substituted, containing solvent plus the light-absorbing molecules whose concentration is to be determined. The decrease in light intensity is noted and the concentration of the solute is calculated from the absorption produced by known concentrations.

The same results can be obtained by the addition to the light path of layers of solution of constant thickness. Such addition can be accomplished by removal of a displacement plate immersed in the solution. By way of example, reference may be had to the drawings, Figure 1 of which shows an ordinary test tube 1, somewhat wider than the beam 3 of monochromatic light, or light of restricted wavelength presented by a spectrophotometer. The tube, containing a solution 4 the optical density of which it is desired to determine, is centered in a stable position in the light beam of the spectrophotometer. A displacement plate 2 shown in longitudinal section through the narrower dimension, and being a rectangular strip of plate glass or similar substance as discussed herein, narrow enough to fit into the tube, and wider than the beam of monochromatic light, is set into the solution in such a way as to be perpendicular to the light beam. The length of light path through the absorbing solution is obviously A plus B. The distance L, being the thickness of the displacement plate, and the plate being made of a material

transparent to light of the wave-length used, does not contribute appreciably to the absorption of the light used. Provision is made in the use of the displacement plate to compensate for this small absorption of light in the distance L.

Figure 2 shows everything shown in Figure 1, in the same position and under the same circumstances, except only that the displacement plate has been withdrawn. The length of light path through the absorbing solution is now A plus B plus L. The change in length of the light path through the absorbing solution is obviously L. This distance is independent of A, of B, and of A plus B. In effect, by removal of the displacement plate, one has interposed into the light path a rectangular layer of absorbing solution of the same dimensions as to width and thickness as the removed displacement plate. Within the limitations discussed elsewhere, the effect of this interposed layer of solution will be independent of the size of, and of irregularities in, the test tube 1. It thus becomes unnecessary to use expensive rectangular optical cells, or carefully selected tubes, to contain the absorbing solution, since one can by using a displacement plate as described always interpose in the light beam a constant, reproducible layer of absorbing solution.

Figure 3 shows a transverse sectional view at the level of the light beam (3—3 in Figure 1), with the displacement plate in position.

For a given solution and wave length the effect of the displacement plate itself on light transmittance will be constant. Either of two procedures can be followed: (1) With the displacement plate immersed at right angles to the light beam, light intensity is set to read 100% (unity). The plate is then removed, and light intensity is read after removal. (2) Light intensity is allowed to remain such that transmittance is close to unity (between 80 and 100%), and transmittance is read exactly, before and again after removal of the displacement plate. The difference in the optical densities corresponding to the two transmittances, corrected by a similar density difference obtained with a blank, gives an optical density corresponding to that of the layer of solution displaced by the plate.

It is possible to use the latter procedure because of the following mathematical relationship:

Let T_0 represent transmittance when the tube without plate contains only solvent, and T_1 and T_2 represent transmittances of the cell containing colored solution with and without the displacement plate, respectively. Then if optical density is represented by D,

$$D_1 = -\log T_1/T_0 = -\log T_1 + \log T_0$$

and

$$D_2 = -\log T_2/T_0 = -\log T_2 + \log T_0$$

The optical density change produced by removing the plate is

$$D_2 - D_1 = -\log T_2 + \log T_1$$

Hence, even though T_0 is much larger than unity (100%), if T_1 and T_2 fall on the scale, it is still possible, from the difference in their logarithms, to obtain the optical density value of the displaced solution.

Provided the cell is not moved during removal of the displacement plate, and provided the latter is positioned in a plane perpendicular to the light beam and is larger than the light beam, the accuracy of results obtained should be dependent only on the precision of determining the change in light density produced by removal of the displacement plate. Hence even scratched test tubes can be used. Furthermore, because the change in density is determined only by the characteristics of the displacement plate, there is no need to select the tubes for exact uniformity of size.

Ideally the plate should be in a plane perpendicular, or normal to the light beam. Rotation of the plate from the plane will produce (1) variation in the reflectance at entrance and exit surfaces, and (2) variation in the length of solution displaced due to the longer slanting path of the light beam through the plate. Factors concerned in these two sources of error are (1) refractive index of the liquid, n_1, (2) refractive index of the displacement plate, n_2 and (3) angle of incidence, i.

Take $n=n_2/n_1$. Also take $E=\sqrt{n^2-\sin^2 i}$ and

$$R=\frac{\text{reflected light}}{\text{incident light}}$$

Fresnel's formula can be modified to give R as a function of n and of the angle of incidence:

$$R=\left(\frac{E-\cos i}{E+\cos i}\right)^2\left(\frac{E^2+\sin^2 i\tan^2 i}{(E+\sin i\tan i)^2}\right)$$

Assuming for n_1 the value 1.33, and for n_2 the value 1.55, n becomes 1.165, and the above formula gives values for fraction of light reflected at the entrance surface, at varying values of i, as shown in Table I under R.

Table I.—Variation in light transmittance with angle of incidence, due to reflection R, and length of light path, L

Angle of Incidence, i	R	L
0°	0.00581	0.0
1°	0.00581	0.000086
2°	0.00581	0.000447
3°	0.00581	0.00101
4°	0.00581	0.00180
5°	0.00581	0.00281
10°	0.00581	0.0113
15°	0.00583	0.0256
20°	0.00588	0.0461

As will be seen, reflection does not change appreciably for small deviations in the position of the displacement plate.

The situation is different, however, with respect to the change in length of the path of the refracted ray through the displacement plate with rotation.

Taking D as the optical density of a layer of the displaced liquid equal in thickness to the displacement plate set normal or perpendicular to the light ray, and taking D_f as the apparent or false optical density of a thicker layer of liquid corresponding to the longer path of the refracted ray through the rotated plate, we can define the resulting positive error, L, as

$$L=\frac{D_f-D}{D}$$

It can be shown, using the notations given above in the determination of reflected light, that

$$L=\frac{D_f-D}{D}=\frac{n-E}{E}$$

Again assuming $n=1.165$, the values for L corresponding to various angles of incidence are given in Table I. It will be noted, if r is taken as the angle of refraction of the light ray corresponding to the angle of incidence, i, that

$$L=\sec r-1$$

Displacement spectrophotometry offers the opportunity for making accurate relative optical density measurements in unselected test tubes, which can be calibrated for certain volumes, used in the water bath, employed for dilution to volume without transfer, and then placed directly in the photometer. Displacement plates have been employed previously in optical cells but only for the purpose of decreasing thickness of the liquid layer, for use with liquids of high optical density. Their use in ordinary test tubes as described here may make desirable readjustment of the concentrations of light-absorbing molecules to secure an optimum optical density range. This change can be produced by using a larger aliquot of the sample, by lesser dilution of the final colored solution, or in some cases by using a different wave length of light at which the absorption is greater.

The maximum thickness of displacement plate that can profitably be used will be determined by several considerations. The plate should be enough wider than the light beam used that stray light will not escape around the edges. Furthermore, the width of the plate should be enough to avoid reflection from the edges. Within the limits of the circular cross section of the tube, the thicker the plate is, the narrower it will have to be.

Because the plate displaces solution, care will have to be taken that liquid is not caused to run over when it is inserted. If dilution to a mark is made, part of the contents can be spilled into the sink after mixing, to reach a safe level. Thickness of the plate will determine the amount of displacement, and hence the amount of liquid that can safely be left in the tube.

The errors in displacement spectrophotometry include in general all those of other types, with one or two exceptions. An exception is the contribution to error made by surface dirt or scratches on the absorption cell surface. These do not, within limits, affect the accuracy of displacement spectrophotometry.

Errors peculiar to displacement spectrophotometry include dirt, scratches, or bubbles on the displacement plate. These imperfections may cause a loss of uniformity if several plates are used. When a single plate is used surface imperfections that are constant in effect, such as scratches, should produce little error, whereas changing imperfections such as bubbles will cause errors even when only a single plate is used.

It can be shown that it is not necessary to achieve precision in setting the plate at right angles to the light beam, but that good accuracy can be attained by approximating the proper position. Sufficient accuracy can be attained by inspection, without elaborate precautions. Out of twenty consecutive times in which the plate was set in place by inspection and in which the angle of deviation of the plate from normalcy was then measured, in no instance did the deviation exceed 2°. At such values of i, the error from this source is negligible.

A more serious source of error is the presence of inequalities in the displacement plates used. Ordinary polished plate glass can vary more than 2% in thickness over very short distances. Glass plates and even polished narrow-band-pass filters may deviate 1% or more in the parallelism of the two surfaces. Such lack of parallelism will affect the integrated thickness of the plate within the light beam. The limits of this variability in commercial plate glass differ with the method of manufacture, and should be least with drawn glass finished with larger polishing blocks. There is a possibility of error arising from local variations in the index of refraction of glass not prepared for optical purposes. These errors can be minimized by using a single displacement plate, which will have to be wiped dry between tubes containing solutions with appreciable differences in concentration. Cleaning would be facilitated by treatment of the surface with a silicone, or by making the plate from a transparent nonwetting plastic of sufficient resistance to chemicals. Care would have to be taken that air bubbles did no cling to such a surface on immersion.

It should not, however, be difficult to prepare plates of glass, plastic, quartz, or other transparent material, of sufficiently uniform thickness and optical characteristics to be interchangeable.

Light absorption of the "unused" or undisplaced solution will act as an additional light filter, and may accentuate stray light effects, especially with wide band width. Thus, marked changes in the geometry of the containing vessel may affect the apparent density values obtained.

Density differences obtained as described are not pro-

portional to concentration of solute. Plotting of density difference values against concentration gives a straight line which in general does not go through the origin. The same statement applies to the usual type of spectrophotometry, unless a blank is used, because of the constant contribution to each value by the "blank" absorption. It is customary to correct for this blank value, either automatically in the setting of the initial light intensity (setting the instrument for $T=100\%$ with a blank), or by subtracting the density value of the blank from each of the other values. In displacement spectrophotometry this constant value is the resultant of the reagent blank plus the contribution of the plate itself (absorption by the glass, reflectance, and other factors). It can easily be determined by measuring the density difference produced by immersion of the plate in a "reagent blank" such as is usually prepared.

Ordinary, unselected test tubes can be used for quantitative absorption spectrophotometry by making density readings before and after removal of a flat plate of glass or other transparent material set in the tube to displace a constant depth of solution. Studies of the nature and magnitude of errors that may be encountered in the procedure show that with moderate precautions good accuracy can be anticipated.

I claim:

In the process of spectrophotometric determination of the concentration of molecules that absorb radiant energy in a fluid of concentration unknown as regards such molecules by the measurement of absorption of radiant energy from a narrow beam of radiant energy of suitably restricted wavelength passed transversely through a substantially transparent container of the fluid, the steps comprising: displacing fluid within said container by inserting a displacement plate with smooth, parallel, plane surfaces, made of a material absorbing little of the radiant energy employed, at right angles across the radiant energy beam to produce within the fluid an optically substantly void space, with smooth, parallel, plane surfaces, transverse to the beam of radiant energy employed and wholly containing the cross section of said beam; measuring the radiant energy transmitted in said beam through said container, displacement plate, and undisplaced fluid; withdrawing the displacement plate, whereby the unknown fluid replaces the space occupied by the plate, thus producing an increase in the length of path of the said beam of radiant energy through said fluid corresponding exactly to the distance the beam previously traversed the optically substantially void plate, and thus causing a greater absorption of the radiant energy used; measuring the radiant energy transmitted in said beam through said container and fluid with said displacement plate removed; the change in transmission of radiant energy being determined by the dimension of the displacement plate along the axis of the beam of radiant energy used, and being reproducible because of the constant dimensions of the displacement plate, and being substantially independent of the shape and optical condition of the surface of the container of the fluid of unknown concentration, said change in transmission of radiant energy making possible calculation of the unknown concentration of energy-absorbing molecules being measured.

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