glass tongs, under the surface of the agar medium. The glass stopper is immediately inserted in the neck of the flask and the flask intermittently shaken until cool. The contents are then poured into 90 by 15 mm. Petri dishes.

(4) Approximately 25 grams of nutrient agar–preservative mixture shall be placed in each Petri dish. Immediately after cooling the culture plates shall be inoculated with the test fungus. The inoculum should be approximately 1 cm. square and should be taken from 14-day-old plates of fungus. The inoculum is placed near the center of the dish and the mycelium side is turned upwards.

(5) The test plates shall be incubated for 14 days at 28° C. The amount of radial growth shall be measured daily for 6 days and every other day from 7 to 14 days. If photographs are taken of the test plates, this should be done on the thirteenth or fourteenth day. When the toxicity

of non-volatile substances is determined, the plates may be placed directly in the incubator. When volatile substances are tested, some method, using either glass-stoppered flasks or bell jars covering the dishes, must be used to reduce the loss of the substance through volatilization.

(6) If no growth occurs on the plates or inoculum after 14 days, the inoculum shall be transferred to standard malt-agar slants to determine if it is dead or alive. In making this transfer, the fungus growth on the inoculum shall be placed in direct contact with the nutrient agar slant. If no growth occurs in 14 days, the inoculum shall be considered to have been killed.

(7) The results of the tests by the above toximetric method shall be stated for both total inhibition and killing point in terms of the percentage of preservative used in the medium.

Quantitative Analysis by Monochromatic Transmission[1]

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A USEFUL derivation from Lambert's law has been developed which enables one to calculate the decimal proportions of the components in a mixture from precise measurements of light-transmission factors.

Derivation of Formula

In its simplest form, where there are only two components in the mixture, the law is expressed as follows:

$$T_m = T_1^{n_1} \cdot T_2^{n_2}$$

in which
T_m = transmission factor of mixture at some particular wave length
T_1 = transmission factor of one ingredient of mixture at same wave length
T_2 = transmission factor of other ingredient of mixture at same wave length
n_1 = decimal proportion of first ingredient in mixture
n_2 = decimal proportion of second ingredient in mixture
$n_1 + n_2 = 1$

To facilitate computation, the formula may be expressed as follows:

$$n_1 = \frac{\log T_m - \log T_2}{\log T_1 - \log T_2}$$

Figure 1 shows the spectral-transmission curves of three liquids: A, yellow potassium chromate solution; B, blue ammoniacal solution of copper; and C, green liquid composed of equal parts by volume of solutions A and B.

Application of Formula

Applying the foregoing formula to the transmission factors at any particular wave length, we secure the results given in Table I, in which the transmission factors of the yellow potassium chromate solution are designated T_1, those of the blue ammoniacal copper solution T_2, and those of the 50–50 mixture as T_m.

Consideration of the curves and of the proportions derived from application of the formula indicate that accuracy is best secured when the calculations are based upon determinations at a wave length in a region where the variations in

the transmission factors are relatively small with large variations in wave length, and at which there are considerable differences between the transmission factors of the components entering into the mixture.

Table I

WAVE LENGTH	TRANSMISSION FACTORS			PROPORTIONS	
mμ	T_1	T_2	T_m	T_1	T_2
				%	%
680	0.882	0.230	0.448	49.6	50.4
660	0.878	0.196	0.410	49.2	50.8
640	0.872	0.175	0.386	49.3	50.7
620	0.866	0.161	0.374	50.1	49.9
600	0.860	0.158	0.372	50.5	49.5
580	0.848	0.170	0.382	50.4	49.6
560	0.830	0.215	0.422	49.9	50.1
540	0.802	0.300	0.495	50.9	49.1
520	0.762	0.412	0.554	48.2	51.8
500	0.628	0.540	0.581	48.5	51.5
480	0.125	0.662	0.290	49.5	50.5

Where there are wide differences in the transmission factors of one of the components at wave lengths not widely separated —as, for example, those of the potassium chromate solution between 480 and 540 mμ, or those of the ammoniacal copper solution between 480 and 560 mμ—a very slight error in the adjustment of the spectrometer will introduce an error which the subsequent calculation will greatly magnify. Similarly, error may be introduced when the calculations are based upon determinations at a wave length where the transmission factors of the components are not appreciably separated. For example, in Table I, if the transmission factor found for the mixture at 500 mμ had been 58.2 per cent instead of 58.1 per cent as recorded, then the proportions found by calculation would have been 49.6 and 50.4 per cent instead of 48.5 and 51.5 per cent as shown. In other words, an error of 0.1 per cent in the measurement of the transmission factor at this wave length resulted in a final error of 1.1 per cent, whereas a similar variation at 560 mμ would have resulted in a change in the final result of only 0.2 per cent.

Accuracy Obtainable

It is essential, of course, for the practical application of this method of quantitative analysis that all measurements of transmission factors be made with a high degree of accuracy, such, for example, as is obtainable with an A. P. C. photo-

electric spectrophotometer with which all measurements are made electrically with a degree of accuracy of about 0.1 per cent. The degree of accuracy obtainable by this method of analysis is illustrated by the following examples.
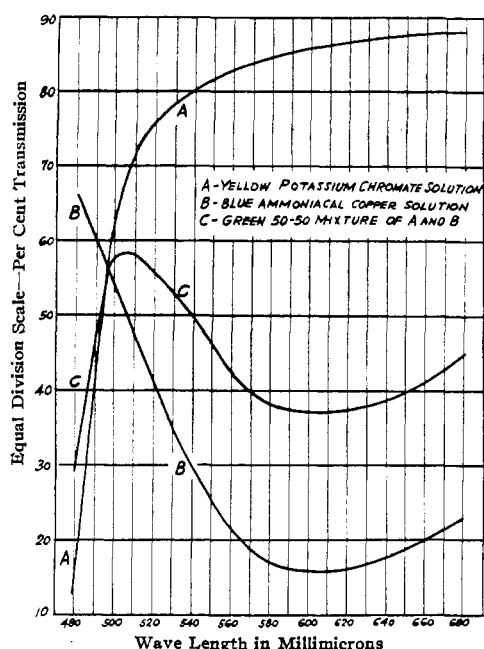

Wave Length in Millimicrons
Figure 1—Spectral Transmission Curves

Three mixtures of peppermint and spearmint oils were prepared—(1) 80–20, (2) 75–25, and (3) 70–30 —and on different occasions, several days apart, determinations were made of the transmission factors of the components and of the three mixtures, and the proportions of the components in each mixture calculated according to the formula previously given. The results are shown in Table II.

Table II

Oils	Transmission at 440 mμ	Oils in Mixture	
		Peppermint	Spearmint
	%	%	%
Peppermint	84.2		
Spearmint	67.2		
80-20 mixture	81.0	82.8	17.2
Peppermint	84.0		
Spearmint	67.75		
80-20 mixture	80.5	80.2	19.8
Peppermint	84.2		
Spearmint	67.2		
75-25 mixture	79.3	73.4	26.6
Peppermint	84.0		
Spearmint	67.75		
75-25 mixture	79.7	75.6	24.4
Peppermint	84.2		
Spearmint	67.2		
70-30 mixture	78.8	70.6	29.4
Peppermint	84.0		
Spearmint	67.75		
70-30 mixture	78.5	68.5	31.5

The averages of the proportions shown in Table II are as follows:

Oils in Mixtures		Oils Found by Analysis	
Peppermint	Spearmint	Peppermint	Spearmint
%	%	%	%
80	20	81.5	18.5
75	25	74.5	25.5
70	30	69.6	30.4

It will be noted that the transmission factors of the oils and of their mixtures varied from day to day. It is believed that such variations result from changes in temperature and/or atmospheric pressure, probably the former because it was ob-

served that the transmission factors of some oils at a particular wave length increased when their temperatures were raised.

Precautions to Minimize Error

Therefore it is advisable, in order to minimize errors due to variations in temperature and pressure, to measure the transmission factors of components and mixtures at room temperature within a few minutes of each other. When, as in the examples given in Table II, all measurements are made at but a single wave length, it is entirely practicable to make the three determinations required for a mixture with only two ingredients in 10 minutes or less.

In actual practice, in order to determine the proportions in a mixture of components of which the transmission characteristics are unknown, the first step is to determine the spectral transmission curve of each component. Thus, in a case such as that illustrated on Figure 1, the contours of curves A and B would be determined. Examination of these curves shows that the zone within which the greatest degree of accuracy is obtainable lies between 590 and 620 mμ, because in this range the variation of curve A is only about 1.2 per cent, and of curve B about 0.4 per cent.

Then the spectrometer would be set at about 605 mμ with the shutter eyepiece adjusted to pass a band about 5 mμ wide. This setting and adjustment would be retained exactly throughout all subsequent measurements. The A. P. C. photoelectric spectrophotometer is then calibrated so that the total energy transmitted by the light source through the spectrometer equals 100 per cent, and then successively the percentage of this total that is transmitted by each component and by the mixture is measured by interposing them between the spectrometer and the photoelectric unit, the readings being made electrically with a Kohlrausch slide-wire bridge.

Of course, if the general contours of the transmission curves of the components in a mixture are known, so that the zone within which the greatest accuracy is obtainable can be established, then, in a case similar to that just described, only three transmission factors would have to be determined, a matter of 10 minutes or less.

It is desirable, in order to minimize the possibility of error, to make all measurements of transmission factors with the same absorption cell always in exactly the same position, so that the light will be transmitted through exactly the same thicknesses of glass and liquid in every case.

Likewise the spectrometer employed must be so constructed or modified that no energy will be transmitted except that having a wave length for which the spectrometer is set and the shutter eyepiece is adjusted. With the cheaper class of spectrometer the error introduced by the transmission of stray light can be minimized by the interposition of filters to intercept light of all wave lengths except those desired.

If these precautions are observed, then the degree of accuracy obtainable is limited only by the sensitivity of the galvanometer, and the accuracy of calibration of the slide-wire bridge.

Determination of Three or More Ingredients

When the mixture is composed of three ingredients, their proportions can be determined by this method only if the spectral transmission curves of two of the components intersect. To determine the proportions of three ingredients in a mixture, the spectral transmission factors of the three ingredients and of the mixture are measured at two different wave lengths, one of which must be that at which the curves of two of the components intersect. The general formula for three ingredients is expressed as follows:

$$T_m = T_1{}^{n_1} \cdot T_2{}^{n_2} \cdot T_3{}^{n_3}$$

From the measurements at the wave length where, for example, the spectral transmission curves of T_1 and T_2 intersect, one determines the proportion of T_3 in the mixture. With this proportion known, the proportions of T_1 and T_2 in the mixture are derived from the transmission factors found at any other wave length.

While it is theoretically possible to determine the proportions of the components in a mixture containing four or more, the practical difficulties in securing the greater required number of precise measurements at different wave lengths is so great as to destroy the practical value of this method of quantitative analysis for such complicated mixtures.

Methods of Testing Mothproofing Compounds[1]

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DURING the past few years, with the development of various preparations to render woolen materials mothproof, the effectiveness of these materials has been tested frequently by chemists with little or no biological training. In working with a living organism one encounters various reactions which vary according to the conditions of the experiment. These reactions and habits have not been fully recognized in all cases. The purpose of this paper is to bring out some of the peculiarities of the webbing clothes moth in relation to the standard methods of testing mothproofing preparations. Although the final answer of the effectiveness of any mothproofing preparation is its ability to stand up under home conditions, it takes a long time to carry out a test under such conditions. In order to accelerate the methods of testing preparations, various artificial tests have been devised. The results of such tests are considered to give a final answer as to the effectiveness in the home.

Half-Circle Test

One of the common tests to determine the mothproofing properties of a compound is that generally known as the half-circle test. This consists of putting into a Petri dish two half-circles of woolen material, one treated and one untreated. A number of larvae of the clothes moth are then liberated in the dish and it is put away under suitable conditions for a period of time. Such a test shows whether the treated piece is repellent to the larvae.

If no further care is taken than indicated above, erratic results may be obtained. First, one must be careful that the treated piece of cloth is cut from the same piece of cloth as the untreated, and is exactly the same in all details. Clothes moths will show a preference for a loosely woven over a tightly woven piece of cloth. They prefer a piece of cloth with a long nap to one with little or no nap. A certain piece of dyed cloth may be mothproof when compared with a sample of undyed woolen cloth, but attractive to moths when compared with a piece of wool dyed with different dyes.

Even with these precautions one can run an experiment, cutting the half-circles from the same piece of cloth, and, without treating either sample, show that they prefer one half to the other half. The writer has run such experiments with a piece of gray-green woolen dress goods. This material is a very fine grade of cloth and has a very fine soft nap on one side. By placing one half-circle with the nap side down and the other with the nap side up, it is possible to concentrate all the feeding on the nap on the under side of the cloth. (Figure 1) The same can be done with mohair. Such an experiment merely indicates that the larvae of the clothes moth like to work between two surfaces. On repeating the mohair

experiment and placing a circle of ordinary muslin over the top of two pieces of mohair, greater injury was obtained on the piece of cloth with the nap side up, indicating that, although they like to work between two surfaces, they prefer working between two surfaces of cloth rather than between a surface of wool and one of glass. The cloth should be placed in the dish so that the moth larvae do not show a preference for one piece because of its arrangement in the dish.

The next precaution deals with the selection of larvae for the test. Clothes-moth larvae cause two types of damage—damage due to feeding, and damage due to the cutting of fibers to be incorporated in the cocoon which they form when ready to pupate. The larvae normally feed upon animal fibers, such as wool and feathers, but when ready to make their cocoons they will cause damage to almost any material available which has suitable fibers for incorporation in these cocoons, whether wool, cotton, silk, rayon, or even paper. The following experiment illustrates this point. A Petri dish is made up consisting of a half-circle of wool and a half-circle with a cotton material having a nice nap. By using partly grown feeding larvae the damage will be confined entirely to the wool. Repeating the experiment in exactly the same way, but using larvae which are practically ready to pupate, damage will result to the cotton due to cocoon formation. (Figure 2) In determining the feeding preferences of clothes-moth larvae, partly grown individuals should be used, since with fully grown larvae erratic results may be obtained. In addition to the size of the larvae, the number employed has a decided bearing. There is a general tendency to use an abnormally large number of larvae in running a test, thinking thereby to accelerate the results. Under natural conditions the heaviest infestation which the writer has ever encountered did not represent more than one larva per square inch. On this basis ten larvae per dish would represent natural conditions.

A material which is repellent to clothes moths will show no damage, even from as high as fifty feeding larvae, over a period of 4 weeks. The same material gave protection against fully grown larvae for 4 days, but when the experiment was run for 3 weeks the nap from the untreated piece was totally destroyed, with the result that when the last larva was ready to form its cocoon the most suitable place was on the treated sample, with the result that one cocoon was formed on the treated portion. The presence of abundant nap, whether treated with a mothproofing preparation or whether present on a material which is not eaten by a clothes moth, is a powerful attrahent for cocoon formation.

It would be well to see how the larvae of the webbing clothes moth react to several different types of materials used for mothproofing purposes. Phenylsalicylate is sometimes used as a moth-proofing agent. When a piece of cloth recently treated with a carbon-tetrachloride solution of phenylsalicylate is placed in a half-circle test with an untreated piece of