

• Technical

Determination of Germicide Mixtures in Soaps and Detergents¹

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A method has been developed for determining qualitatively and quantitatively all commonly used soap germicides. It includes the halogenated salicylanilides, halogenated carbanilides and bisphenols both alone and in mixtures. Elimination of interfering substances is accomplished by extraction with dimethylformamide followed by evaporation to dryness. The residue is then taken up in both acidic alcohol and basic alcohol. The ultraviolet patterns obtained under these conditions are typical of the various germicides and are used to identify them.

THE USE of germicidal agents in soap has become increasingly popular during the last ten years. It is estimated that at present in excess of 40% of all the toilet bars sold in the United States contain bacteriostatic additives.

Only a few types of organic compounds have proved to be suitable for incorporation into soap as germicides. They are used either alone or as synergistic mixtures. In the latter case, increased effectiveness of the mixture over the effectiveness of the sum of the individual germicides has been demonstrated (9). Bisphenols, such as 2,2'-methylene bis(3,4,6-trichlorophenol), the corresponding dichloro and tetrachloro analogues and 2,2'-thio bis(4,6-dichlorophenol) were some of the earliest germicides used in soap. Halogenated salicylanilides are a second class of compounds widely used for this purpose. Two other effective germicides for soap are 3,4,4'-trichlorocarbanilide and tetramethylthiuram disulfide.

Several methods have been reported in the literature for the analysis of soap germicides (1-8, 10, 11). Most are based either on ultraviolet spectroscopy or on colorimetric methods in which a color is obtained by reaction of an active functional group with a suitable reagent. In general the published methods suffer from two limitations:

1. Perfumes or other materials in the soap often interfere.

2. The lack of specificity usually requires more than one test for the determination of all the possible soap germicides. The method reported in the present paper overcomes both these difficulties and is also applicable to mixtures.

Experimental

Reagents: (a) Dimethylformamide, spectral grade. (b) Acidic alcohol. Prepared by mixing 96 ml. of 95% ethanol with 4 ml. of glacial acetic acid. (c) Basic alcohol. Prepared by mixing 96 ml. of 95% ethanol with 4 ml. concentrated ammonium hydroxide.

Instrument: Beckman model DU Spectrophotometer with ultraviolet light attachment.

Procedure: A 10 g. sample is shaved into an Erlenmeyer flask and 100 ml. of dimethylformamide are added. The mixture is stirred at room temperature for 15 min. after which it is filtered or centrifuged. Two 1-ml. aliquots are evaporated to dryness over the steam bath. One residue from evaporation is taken up in 100 ml. of acidic alcohol and the other residue is taken up in 100 ml. of basic alcohol.

Using a Beckman DU Spectrophotometer with a hydrogen lamp attachment, transmission vs. wavelength is plotted for both the acidic and basic alcoholic germicidal solutions from 220 to 400 m μ wavelength. Quantitative determination of germicides is made by referring to a standard extinction curve for each specific compound.

Results and Discussion

The method of determining germicides in the presence of soap described above is dependent upon several factors. a) Dimethylformamide is used as the extraction solvent because soap and inorganic salts are not appreciably soluble; the solution obtained contains mainly germicide, color and perfume components. All commonly used germicides are readily soluble in dimethylformamide; rapid extraction is, therefore, effected. b) Evaporation of aliquots is done on a steambath since steam temperature will not decompose the germicide but frequently destroys organic dyes. Under these conditions, most of the perfume components are also driven off. This removal of perfume and dye is an important part of the method since frequently these compounds possess ultraviolet spectra which interfere with the germicide determination itself. c) Dissolution of the residue from evaporation is made with alcohol to allow measure-

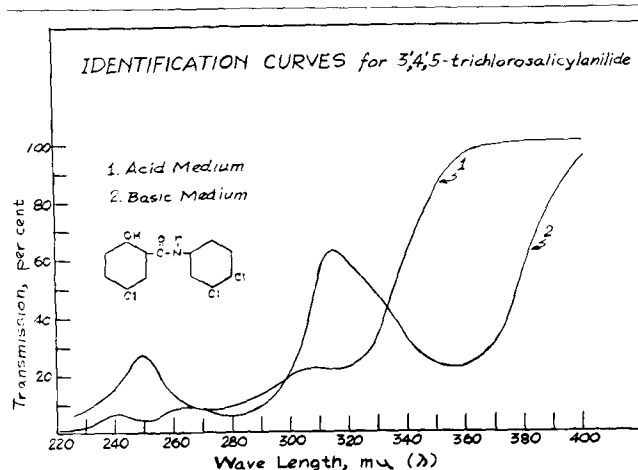


FIG. 1. Identification curves for 3',4',5-trichlorosalicylanilide.

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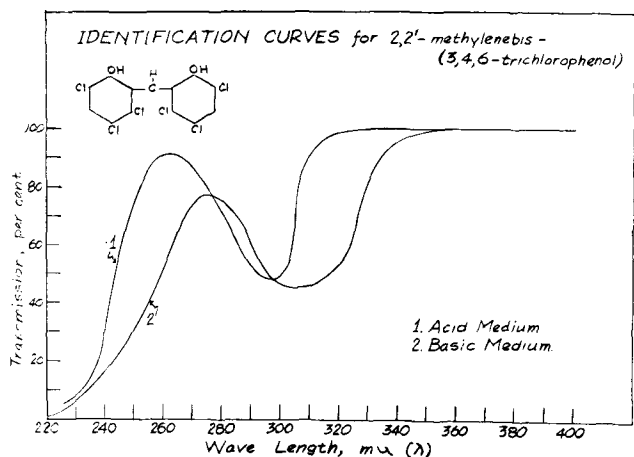


Fig. 2. Identification curves for 2,2'-methylene bis(3,4,6-trichlorophenol).

ments down to 220 $m\mu$. Dimethylformamide has total absorption below 267 $m\mu$. Alcohol is not used as the original extraction solvent because of the high solubility of soap in this medium. d) Two different levels of pH are used because germicides which contain a phenolic group will exhibit different spectra depending upon the degree of ionization of the phenol group and the resulting electron cloud shifts associated therewith. e) Extinction curves are prepared from both acidic and basic solutions and, if possible, at more than one wavelength for each curve. This has the effect of shifting the spectrum of phenolic germicides away from that of possible interferences. Also, it is highly unlikely for any interference to have an absorption spectrum parallel to the spectrum of a germicide. If measurements at several wavelengths do not result in identical values for absorption, interference is present and the higher values are disregarded.

The patterns obtained from the various classes of germicides are used as a means of identification.

Salicylanilides: The absorption curve of 3',4',5-trichlorosalicylanilides from 220 to 400 $m\mu$ is shown in Figure 1. The pattern obtained is typical of salicylanilides as a group. The major contribution to the spectra in this region is the general molecular configuration and not specific substitution. Variation in the halogen substituents have little effect and the three salicylanilides reported in this paper possess almost identical spectra. However the substituents do make a small but sufficient spectral contribution which can be used to distinguish the three, namely the 3',4',5-trichloro, the 3,5,4'-tribromo, and the 3,3',4',5-tetrachloro derivative. This is done by examining the isobestic points of these compounds. These points are the wavelengths at which, in this case, the acidic and basic forms have equal extinction. The values of the isobestic points for the three halogenated salicylanilides mentioned above are 333, 343, and 340 $m\mu$, respectively. These values are sufficiently apart to permit differentiation of the three salicylanilides.

Bisphenols: The identification curve for 2,2'-methylene bis(3,4,6-trichlorophenol) is shown in Figure 2. This bisphenol exhibits the typical phenolic shift when subject to changes in pH. In the case of the bisphenols, substituents have a much greater effect on the spectrum than with the salicylanilides and very little

trouble is encountered in distinguishing between them. This is shown in Figure 3, where both the acid and basic forms of 2,2'-methylene bis(4-chlorophenol), 2,2'-methylene bis(3,4,6-trichlorophenol) and 2,2'-thio bis(4,6-dichlorophenol) are compared.

Non-Phenolic Germicides: The typical curve for trichlorocarbanilide is shown in Figure 4. Here, identical curves are obtained in both the acidic and alkaline media above 240 $m\mu$. On the other hand, the "fishtail" split occurring below 240 $m\mu$ is characteristic for the carbanilides and can be used for their identification.

A similar type curve is obtained with tetramethylthiuram disulfide. Here again changing pH does not cause a shift in the spectrum. Distinction between the thiuram spectrum and carbanilide spectrum is that the former does not have the "fishtail" at low wavelengths but absorbs almost completely below 290 $m\mu$.

Mixtures of Germicides: Where a soap bar contains a mixture of germicides the curve obtained contains contributions from each of the compounds present. Figure 5, curve 1 represents the pattern obtained in basic medium from a mixture of equal parts of tetramethylthiuram disulfide and 3',4',5-trichlorosalicylanilide. The part of the curve from 310 to 390 $m\mu$ is contributed by the salicylanilide. The absorption at 360 $m\mu$ is typical of the salicylanilides and disappears in acid medium demonstrating the characteristic spectral shift of the salicylanilide. The curve from 220 to 300 $m\mu$ comes primarily from the tetramethylthiuram disulfide and, as expected, remains the same regardless of pH. An example of a mixture where the absorbing power of the two compounds is not of the same order of magnitude is shown in Figure 5, curve 2, which represents 3,4,4'-trichlorocarbanilide in equal concentration with 2,2'-methylene bis(3,4,6-trichlorophenol). Most of the curve is contributed by the 3,4,4'-trichlorocarbanilide since its absorbing power is ten times that of the other germicides; how-

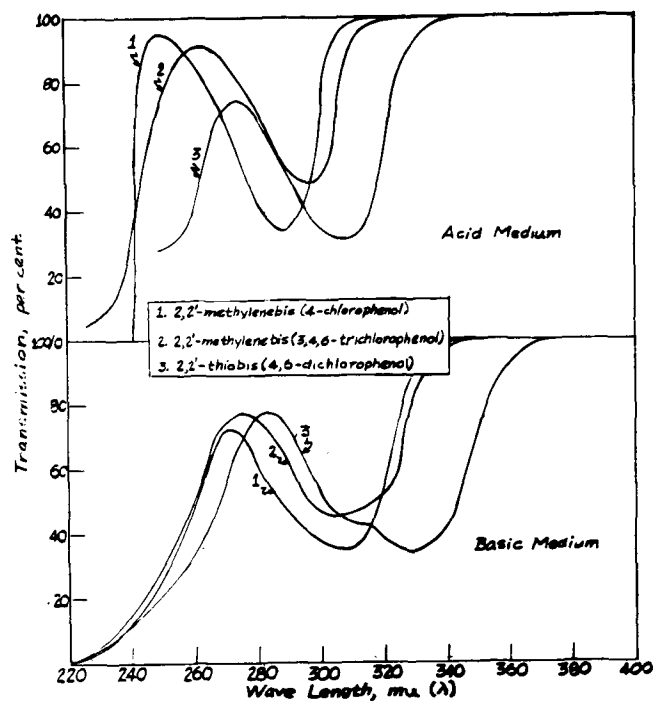


Fig. 3. Comparison of acidic and basic spectra of three bisphenols.

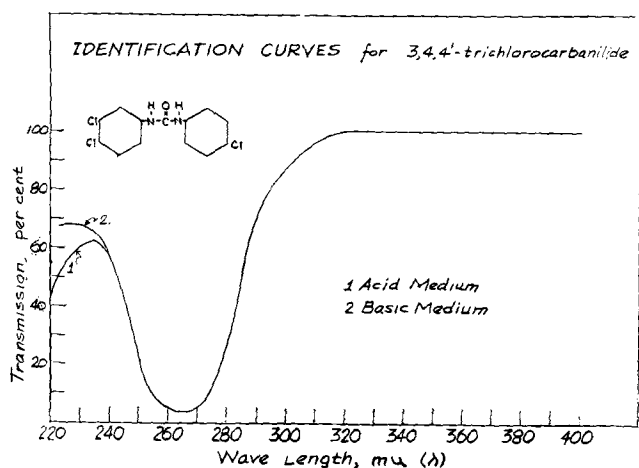


FIG. 4. Identification curves for 3,4,4'-trichlorocarbanilide.

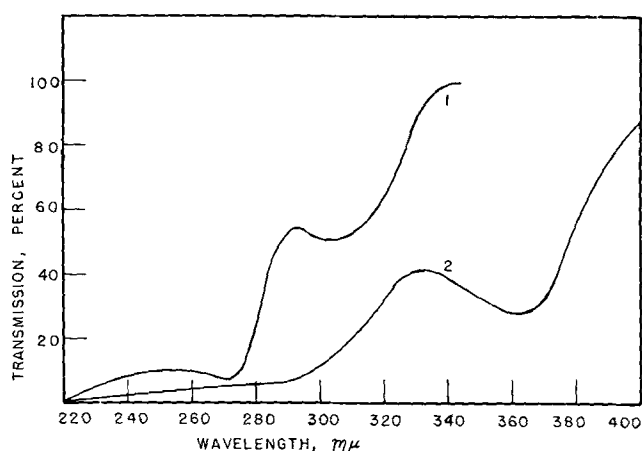


FIG. 5. Absorption curve of a mixture containing tetramethylthiuram disulfide and 3',4',5-trichlorosalicylanilide (curve 1) and absorption curve of a mixture containing 3,4,4'-trichlorocarbanilide and 2,2'-methylene bis(3,4,6-trichlorophenol), (curve 2).

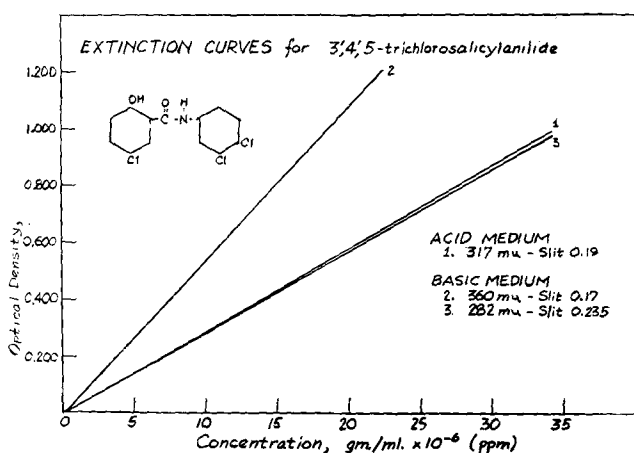


FIG. 6. Extinction curves for 3',4',5-trichlorosalicylanilide.

ever it can be seen that another compound is present. Increasing the total concentration emphasizes the portion of the pattern contributed by the 2,2'-methylene

bis(3,4,6-trichlorophenol) but at the same time, contributions from both compounds produce total absorption at lower wavelengths.

Quantitative Determination of Germicides: Having determined the germicidal agents present in the soap, the amount is readily determined by taking readings at the appropriate wavelength. A typical extinction curve, this particular one being for 3',4',5-trichlorosalicylanilide, is shown in Figure 6.

The values of optical density are determined at several wavelengths and under both acidic and basic conditions; therefore, if interference is being encountered, it will be obvious by the fact that one or more readings will give an apparent concentration which is too high. In such cases the lowest value is the one contributed solely by the compound in question without contribution from extraneous sources. The wavelengths at which extinction measurements are made under both acidic and basic conditions are listed in Table I.

TABLE I
Extinction Maxima

Compound	Wavelength	
	Basic	Acidic
3,4,4'-Trichlorocarbanilide.....	265	265
3',4',5-Trichlorosalicylanilide.....	360, 282	317
3,3',4',5-Tetrachlorosalicylanilide.....	360, 282	317
3,5,4'-Tribromosalicylanilide.....	360, 282	317
2,2-Methylene bis(3,4,6-trichlorophenol).....	305	296
2,2'-Methylene bis(4-chlorophenol).....	308	287
2,2'-Thio bis(4,6-dichlorophenol).....	330	307
N,N,N',N'-Tetramethyl thiuram disulfide.....	280	280

Application of Procedure: Application of this procedure to identify and determine germicidal agents has been practiced successfully in our laboratory. Samples examined have included both domestic and foreign bar soaps, soap-detergent combinations and detergent powders. In no case has it been impossible to circumvent interference from other absorbing substances. Likewise, the procedure is readily suitable for routine use such as in quality control. The method is also readily applicable to mixtures by choice of suitable wavelengths and dilutions.

Acknowledgment

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