

TABLE III.—PHYSICAL PROPERTIES OF SOME SUPPOSITORY BASES DURING 120-DAY OBSERVATION

Days		0.1	1	10	20	30	60	120
Theobroma Oil, (heated at 35°C.)	CMT	32.2	31.6	33.6	34.3	34.9	35.0	34.6
	LT		34	34	34	34	35	35
	ST		30	30	30	30	32	32
Theobroma Oil, (heated at 55°C.)	CMT	25.1	27.5	27.7	28.0	32.8	32.4	32.0
	LT		29	29	29	30	34	34
	ST		24	24	24	24	24	30
Dehydag III	CMT	33.3	33.7	35.0	35.0	35.5	36.6	36.9
	LT		36	37	37	38	39	39
	ST		35	35	35	36	37	37
Estarinum A	CMT	32.7	34.7	36.7	36.8	37.5	37.0	36.6
	LT		37	38	38	38	38	37
	ST		31	32	32	31	30	31
Estarinum BB	CMT	32.8	34.7	35.2	35.9	36.7	37.1	37.0
	LT		36	36	36	37	37	37
	ST		32	32	31	30	31	32
Imhausen H	CMT	34.4	34.4	34.6	34.7	34.4	36.7	37.0
	LT		35	36	37	37	37	37
	ST		32	32	32	31	29	29
Imhausen W	CMT	34.2	34.8	35.9	36.0	36.4	36.8	36.5
	LT		36	36	36	37	37	37
	ST		31	31	29	28	28	28
Suppocire A	CMT	34.4	34.8	35.9	36.0	36.5	36.8	36.5
	LT		36	36	36	37	37	37
	ST		31	31	29	28	28	28

bases of Table II with a liquefaction temperature below 37° liquefy in rectal conditions as shown by the results reported by Setnikar and Fantelli (1). But this is not necessarily true for fatty bases with a capillary melting temperature below 37°.

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Proposed Method of Assay for Diaphene

By SOBHI A. SOLIMAN and LOYD E. HARRIS

A rapid and convenient differential spectrophotometric assay is proposed for the assay of Diaphene in liquid soaps and hand creams.

DIAPHENE¹ is a mixture of 5,4'-dibromosalicylanilide and 3,5,4'-tribromosalicylanilide, and is used in toilet detergent formulations. It is reported to possess an unusually long-lasting germicidal effect.

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¹ Trademark of Norton Co., Worcester, Mass. Contributed and marketed by Stecker Chemicals, Inc., Ridgewood, N. J.

Methods described in the literature for the determination of phenolic antiseptics in soaps employ colorimetric or spectrophotometric techniques. The method of Gottlieb and Marsh (1), depending on color formation by the reaction of a phenol with 4-aminoantipyrine in the presence of potassium ferricyanide as an oxidizing agent, was reported to give unreliable results since the color fades rapidly. Ettinger, *et al.* (2), in an attempt to stabilize the color, concentrated the reaction products by extraction with chloroform. This modification, however, does not stabilize the color produced to any degree of accuracy.

The colorimetric method of Johnson and Savidge (3) depends upon the measurement of the

color produced by the coupling of the phenol with 4-aminophenazone in the presence of an alkaline oxidizing agent. The method was reported to give unsatisfactory results with hexachlorophene and dichlorophene since the darkening characteristic of these substances in alkaline medium considerably changes the orange color.

The method of Larson (4), depending on the color determination of the phenol-ferric chloride salt, involves precipitation of the soap with barium bromide. The method was discussed by Childs and Parks (5) who found that the fleeting color produced in this method made it difficult to obtain reproducible results.

The rapid spectrophotometric method of Lord, *et al.* (6), is applied directly to the soap sample by dissolving it in 0.1 *N* potassium hydroxide solution and measuring its absorbance. Measurements were taken at three different wavelengths in the region of the peak of absorption. A correction for irrelevant absorption due to the soap base is made by application of the Morton-Stubbs three-point geometrical correction.

The spectrophotometric procedure of Clements and Newburger (7) is designed for the determination of hexachlorophene in all types of cosmetic formulations and involves several extractions which would appear to be unnecessary for the determination of its content in a liquid soap.

A differential rapid spectrophotometric method was developed by Childs and Parks (5) for the determination of hexachlorophene in the presence of liquid soap. The advantages of this method lie in the fact that the extraction of the phenolic antiseptic from the soap is not necessary; also, it does not depend upon color determination. The method depends on a substance of equal concentration in both the solute and solvent cells of the spectrophotometer when an absorption is obtained that is essentially the same as a cell blank curve. It appeared that such a technique might eliminate the error caused by absorption of the soap base by allowing the instrument to cancel out the irrelevant absorption.

Alkali increases the intensity of absorption of phenols by converting them from the molecular to the ion or salt form, and shifts their absorption peaks to longer wavelengths. Childs and Parks (5) showed that by determining the absorption of hexachlorophene in alkaline media in the solute cell against its absorption in the acid media in the solvent cell, an absorption curve could be obtained which is unique for the phenol and without interference from the soap base. This technique of determining the absorbance of the phenolic compound at an alkaline pH in the solute cell *versus* its same concentration at an acid pH in

the solvent cell is known as the differential method.

The differential spectrophotometric procedure has been modified by Matuszak, *et al.* (8), in order to be applicable for the determination of bithionol in liquid soap.

Van Der Pol (9) modified the differential method of Childs and Parks (5) so that it may be used for a wide variety of preparations.

The ion exchange resin method of Suffis and Dean (10) for assaying chlorinated phenolic compounds in soaps depends on separating the antiseptic from the soap and then assaying spectrophotometrically. The separation is based upon the fact that chlorinated phenolics are stronger acids than nonchlorinated phenolics and carboxylic acids. Therefore, they may be separated by their differing adsorptive properties on a strongly basic anionic exchange resin. An empirical calibration must be made using soap preparations of known chlorophenolic content. Apparently resin hold-up prevents a quantitative elution, but a partial and reproducible recovery of chlorophenol is obtained.

The ion exchange resin method was later modified (11) to be applicable for the determination of Diaphene in soap.

EXPERIMENTAL

Apparatus and Reagents.—Beckman DU quartz spectrophotometer with matched 1.0-cm. silica cells; methanol, absolute, reagent grade; 90% methanol, prepared by mixing 100 ml. of distilled water with sufficient anhydrous methanol to make 1000 ml.; hydrochloric acid, approximately 0.1 *N* in distilled water; sodium hydroxide, approximately 0.1 *N* in distilled water.

Development of a Standard Diaphene Spectrophotometric Graph.—Differential absorption spectra of Diaphene (3 mg./100 ml.) in 90% methanol were prepared by measuring the differential absorbance (pH 3 *vs.* pH 8) at various wavelengths. The absorption peak was at 280 μ . Ninety per cent methanolic solutions of Diaphene (not more than 5 mg./100 ml.) followed Beer's law at this wavelength. The differential absorption spectrum of Diaphene in the presence of soap exhibits a flat peak at 280, 281, and 282 μ .

Diaphene² was assayed at 280 μ , using the standard absorption graph, in the presence of soap.

A 0.3 *N* acetic acid solution in 90% methanol, was prepared by dissolving 18 Gm. glacial acetic acid in 90% methanol to produce 1000 ml. The solution was then adjusted to pH 3 with 0.1 *N* hydrochloric acid using the Beckman Zeromatic pH meter.

Ninety per cent methanol was adjusted to pH 8 with 0.1 *N* sodium hydroxide using the Beckman Zeromatic pH meter.

Proposed Spectrophotometric Assay for Diaphene.—The differential absorbance index of

² ASC-4 brand, marketed by Stecker Chemicals, Inc.

Diaphene in the presence of soap was determined in absorbance units per mg. per ml. as follows: dissolve 5 ml. of the soap base in 90% methanol to make exactly 100 ml.; dissolve a 0.1000-Gm. sample of Diaphene in absolute methanol to make exactly 100 ml.; and dilute a 50-ml. aliquot of this solution to exactly 100 ml. with 90% methanol (solution A).

Place 5 ml. of the soap base solution in each of four 100-ml. beakers. To each of the four beakers add 2.0, 3.0, 4.0, and 5.0 ml. of solution A. Then to each of the beakers add 20 ml. of 90% methanol and sufficient 0.1 *N* hydrochloric acid to bring the solution to pH 3 on the Beckman Zeromatic pH meter. Wash the electrodes with pH 3 acetic acid-methanol solution. Transfer the beaker contents quantitatively to a dry 50-ml. G. S. volumetric flask, adjust to volume with the acetic acid-methanol solution, and mix thoroughly. This gives a series of solutions containing 2.0, 3.0, 4.0, and 5.0 mg./100 ml. of Diaphene, respectively, at pH 3.

Prepare a second series of solutions of the same concentration as above at pH 8 as follows: Place 5 ml. of the soap base solution in each of four 100-ml. beakers, to each of which add 2.0, 3.0, 4.0, and 5.0 ml. of solution A, then 20 ml. of 90% methanol, adjust to pH 8 on a Beckman Zeromatic pH meter using 0.1 *N* sodium hydroxide or 0.1 *N* hydrochloric acid, in distilled water, as required. Wash the electrodes with 90% methanol at pH 8. Transfer the beaker contents quantitatively to a dry 50-ml. G.-S. volumetric flask, adjust to volume with 90% methanol at pH 8 and mix thoroughly. This gives a series of solutions containing 2.0, 3.0, 4.0, and 5.0 mg./100 ml. of Diaphene, respectively, at pH 8.

Arrange the two series of solutions in pairs of the same concentration. Place the pH 3 member of each pair in the solvent cell of the spectrophotometer and the pH 8 member in the solute cell.

Divide the observed absorbance by the number of mg. of Diaphene per ml. of solution to get the absorbance index, *I*.

The average absorbance index at 280 $m\mu$ and slit width of 1.62 mm., as determined by the differential method, should have a value of 10 absorbance units per mg. per ml. in the presence of soap.

TABLE I.—ABSORBANCE INDEX OF DIAPHENE IN 90% METHANOL CONTAINING SOAP SHAMPOO BASE pH 3 vs. pH 8^a

Diaphene, mg. per 100 ml.	Absorbance	Absorbance Index	Average Absorbance Index
2	0.204	0.204	10.20
3	0.300	0.300	10.00
4	0.398	0.400	9.97
5	0.490	0.495	9.86
			10.00

^a Wavelength, 280 $m\mu$; slit width, 1.62 mm.

Assay Procedure for Diaphene in Liquid Soap.—

Dissolve 5 ml. of the soap sample to be assayed in 90% methanol to make exactly 100 ml. (solution A). The amount taken could be decreased or increased, but the final dilution should not contain more than 5 mg. Diaphene/100 ml.

Place 5 ml. of solution A in a 100-ml. beaker, add 20-ml. of 90% methanol and sufficient 0.1 *N* hy-

drochloric acid to bring the solution to pH 3 using the Beckman Zeromatic pH meter. Wash the electrodes with pH 3 acetic acid-methanol solution. Transfer the contents of the beaker quantitatively to a dry 50-ml. G.-S. volumetric flask, adjust to volume with acetic acid-methanol solution and mix thoroughly.

Place another 5 ml. of solution A in a 100-ml. beaker, add 20 ml. of 90% methanol, adjust to pH 8 on a Beckman Zeromatic pH meter. Wash the electrodes with 90% methanol at pH 8. Transfer the beaker contents quantitatively to a dry 50-ml. G.-S. volumetric flask, adjust to volume with 90% methanol at pH 8 and mix thoroughly.

Measure the absorbance at 280 $m\mu$ and slit width 1.62 mm. by placing the pH 3 solution in the solvent cell of the Beckman DU spectrophotometer, and the pH 8 solution in the solute cell.

The per cent of Diaphene was calculated using the following formula:

$$\text{per cent of Diaphene} = \frac{A \times 50 \times 100}{I \times V \times W \times 100}$$

where *A* = differential absorbance measured; *I* = differential absorbance index (10); *V* = volume of solution A in 50 ml. of the assayed solution; *W* = volume of the sample in ml. \times 1000 or weight in mg.

Soap samples to which known amounts of Diaphene had been added were assayed by this method.

Results are given in Table II. The method was also used for the assay of Diaphene in hand cream³ with results in the same Table.

TABLE II.—DIAPHENE ASSAY IN SOAPS AND CREAMS

	% Diaphene Found
Soap shampoo base plus 0.40% w/v added Diaphene ^a	0.408
Soap shampoo base plus 0.60% w/v added Diaphene	0.602
Soap shampoo base plus 0.80% w/v added Diaphene	0.790
Peck's antiseptic soap base plus 0.70% w/v added Diaphene	0.700
G. S. 40% soap base plus 0.50% w/v added Diaphene	0.502
Hand cream labeled to contain 0.05% w/v Diaphene	0.052
Hand cream labeled to contain 0.05% plus 0.20% added Diaphene	0.253

^a Diaphene added to the 5-ml. aliquot of the final dilution of the sample.

The Effect of Light and Time on Diaphene.—

The effect of sunlight, short ultraviolet, and long ultraviolet light on Diaphene has been studied. The absorbance of 2.5 mg./100 ml. of Diaphene in 90% methanol, containing G. S. 40% soap, at pH 8 was found to decrease gradually on exposure to sunlight for 10 minutes. After that period, the absorbance of the same solution did not show an appreciable change when the solution was exposed to sunlight for a total period of three hours. The solution assumed a distinct yellow color during the 10-minute exposure.

When an acidic solution at pH 3 containing the

³ In Cuticura Laboratories.

same amount of Diaphene and soap was similarly treated, no significant change in absorbance or color took place.

On exposing a similar solution at pH 8 in a 1.00-cm. silica cell to short ultraviolet light (using a short-wave ultraviolet lamp, model SL 2537, with a short-wave ultraviolet filter, Model SL 2537⁴) a considerable decrease in absorbance took place in a period of 40 minutes after which a gradual change occurred through a total period of three hours. When a pH 3 solution was exposed to the short ultraviolet light, no significant change in absorbance took place in the same period of time.

A long-wave ultraviolet lamp, model SL 3660, with a long-wave ultraviolet filter, model SL 3660⁴, was used to determine the effect of long ultraviolet light on Diaphene solutions of pH 8 and pH 3. Absorbances were determined in the same manner (using 90% methanol at pH 8 and pH 3, respectively, in the solvent cell). It was found that long ultraviolet light had more or less the same effect as sunlight on both solutions of Diaphene. No apparent color change took place in either solution after exposure to short or long ultraviolet light, as observed in the 1.0-cm. silica cell. It could be recommended that Diaphene containing preparations that are alkaline should be protected from sunlight as well as ultraviolet light.

DISCUSSION

The spectrophotometric method proposed by Childs and Parks (5) for assaying hexachlorophene in the presence of soap when used for Diaphene gave results that were lower than expected. When liquid soap bases were employed in constructing the standard curve, results were found to be more accurate.

The proposed method eliminates the necessity of the establishment of a standard graph. The

⁴ Mineralight, marketed by Fisher Scientific Co.

method also does not necessitate the presence of the same soap sample free from Diaphene. The proposed spectrophotometric assay for Diaphene was used for assaying liquid soaps and hand creams with accuracy.

SUMMARY

A rapid, convenient, and accurate differential method for the assay of Diaphene in liquid soaps and hand creams has been proposed. The method is based on the differential spectrophotometric assay for hexachlorophene in liquid soaps developed by Childs and Parks (5).

This proposed method does not necessitate the establishment of a standard graph as was necessary in the Childs and Parks procedure. The method also does not require the same soap base free from Diaphene.

The optical density of Diaphene in solutions at pH 8 is reduced by sunlight and ultraviolet light.

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Use of 3-Azabicyclo[3.2.2]nonane in the Mannich Reaction II. Secondary γ -Amino Alcohols

By C. DEWITT BLANTON, Jr., and W. LEWIS NOBLES

The syntheses of a group of secondary γ -amino alcohols by reduction of the corresponding Mannich base with sodium borohydride are described. These alcohols are to be screened for possible pharmacodynamic activity.

IN THE FIRST paper of this series (1), a number of substituted β -amino ketones were synthesized for pharmacological evaluation employing the Mannich reaction and 3-azabicyclo[3.2.2]-nonane as the amine moiety. Previously, Den-

ton (2) and his associates had similarly prepared certain amino ketones and converted them to the corresponding secondary and tertiary alcohols. In many cases, the conversion to the alcohol had a significant effect on the physiological activity. Therefore, it was considered that the transformation of the ketones previously reported by us (1), might possibly have a similar effect. In

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