

Determination of Hexachlorophene Content of Soaps Using Ferric Chloride Reagent

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EXTENDED use of deodorant soaps is expected to result from the property of hexachlorophene to produce a soap with acceptable bactericidal properties. The problem of its content in soap is of utmost importance because of its relatively high cost and the quantity required for activity.

The ferric chloride method described here is rapid and has consistently given excellent precision during a long period of time. It consists of the development of the ferric chloride salt of hexachlorophene, apparently FeCl_2OR^3 , in alcohol solution under controlled conditions of time and temperature. By adding a methyl alcohol solution of barium bromide to the ethyl alcohol soap solution, the soap is precipitated in a fine crystalline form and the hexachlorophene is distributed almost completely into the large alcohol phase.

The method usually used for estimating the hexachlorophene [*bis* (3,5,6-trichloro-2-hydroxyphenyl) methane] content in soap is that of Gottlieb and March (2). This is a quantitative adaptation of Emerson's (1) 4-aminoantipyrine-phenol-potassium ferricyanide reaction, and it is the only quantitative method that could be found in the literature for hexachlorophene. In our laboratory we have found that the results obtained by this method are not reliable. Two other laboratories we have consulted on this method had independently come to this same conclusion.

Barium soaps of the usual mill soap fatty acids are only slightly soluble in methyl-ethyl alcohol at 25°C., the usual solubility being about 0.30 to 0.50 volume percentage, as determined by titration of the filtrate with 0.1 N acid, using bromphenol blue indicator. This concentration is not high enough to cause any difficulty in the analysis. If about 10% or more abietic acid is present in the fatty acid composition however, difficulty will be encountered due to the high solubility of barium abietate in alcohol causing a ferric soap to precipitate during the color development. Cooling to 2°C. will greatly reduce the barium abietate solubility so that 15% of abietic acid can be tolerated in the fatty acids without an insoluble ferric soap forming or necessitating a change in the standard curve. Any barium soap solubility under a maximum of about 0.80 volume percentage will give the same result, and consequently no error is produced by different fatty acid formulae. Sodium abietate is the only common soap that will give abnormally high soap solubilities, and such solubilities are readily detected when the solution becomes cloudy with ferric chloride reagent.

Bar soaps containing hexachlorophene frequently contain other ingredients such as coloring matter, pigments, preservatives, perfume, and glycerol. None of these produces any significant error in the result with sodium soap. A perfume content of 1.5% is not uncommon in these soaps, and the perfumes are invariably a blend of many essential oils, some of which are phenolic in nature, such as eugenol, isoeugenol, vanillin, and amyl salicylate. With this ferric chloride method the eugenols and vanillin do not produce

TABLE I
Solubilities of Various Barium Soaps in Methyl-Ethyl Alcohol

Fatty acid composition	@ 25°C.		@ 2°C.	
	Colorimeter reading	Volume, % anhyd. soap	Colorimeter reading	Volume, % anhyd. soap
100% coconut oil.....	298	0.47
85% tallow, 15% coconut.....	297	0.29	298	0.18
98% tallow, 2% rosin.....	300	0.40
90% tallow, 10% rosin.....	Hazy	0.83	296	0.68
85% tallow, 15% rosin.....	298	0.87
100% rosin.....	Very cloudy	3.6
100% Staybelite ^a	Very cloudy	1.97	Very cloudy	2.09
90% soya oil, 10% coconut oil potash.....	310	0.52
90% soya acids, 10% coconut oil potash.....	310	0.51	315	0.28
100% cocoa, potash.....	310

^aA partially hydrogenated rosin.

any error even when present in an abnormally high concentration in the soap. Amyl salicylate however will produce an error if a large amount is present, but its concentration in perfumes is invariably low enough so that errors from it are less than 0.05%. Pigments and preservatives do not produce any error as they are insoluble and present in insignificant amounts. Glycerol increases the apparent hexachlorophene content 0.06% for each 1% of glycerol present in the soap. Milled sodium soaps however usually contain less than 1% glycerol so that the error introduced from it is usually insignificant and no correction is needed.

High acidity or slight amounts of free alkali in the soap does not produce any error because of the buffering action of the ferric chloride reagent.

Apparatus

1. A precision photoelectric colorimeter or spectrophotometer.
2. Absorption cells.
3. Thermometer, 0-65°C. graduated in 0.2°C. intervals.
4. Constant temperature bath @ 25.0°C. An insulated, 2-liter beaker one-half full of water serves very well. Control the temperature by adding hot or cold water as needed.
5. Volumetric flasks, 50 ml. and 100 ml. Pyrex brand glass.
6. Total delivery pipettes, 1, 5, and 15 ml.
7. Stop watch.
8. Funnels, Whatman's No. 1, 15 cm. filter paper, 50 ml. cylindrical graduate, wire gauze, and hot plate.

Reagents

1. Methanol, anhydrous.
2. 95% ethyl alcohol, No. 3A, No. 30, or No. 40.
3. Ferric chloride solution, 2,500 grams C. P. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ per 100 ml. in distilled water. Use a Pyrex brand glass volumetric flask and prepare fresh solution every two or three days.
4. Barium bromide solution, 50 grams C. P. $\text{BaBr}_2 \cdot 2\text{H}_2\text{O}$ per 500 ml. in methanol.
5. Hengar granules.
6. Hexachlorophene alcohol solution, 1 ml. = 0.00500 grams.

Preparation of Standard Curve

Weigh 5.00 ± 0.05 grams of milled soap chips or any milled toilet soap into each of seven Pyrex brand

TABLE II
 Effect of Perfume Phenolics on Hexachlorophene Analysis

Soap	Type perfume	Phenolics present in perfume, %	Perfume phenolics in soap, %	Color reading	Apparent hexa. content, %	Actual hexa. content, %	Error due to perfume, %
Mill soap base + 1.6% hexa.	Fougere	Amyl salicylate 8.3	0.16	198	1.64	1.60	+0.04
Mill soap base + 1.6% hexa.	Carnation	Amyl salicylate 5.0 Clove 31.0 Eugenol 14.0	0.90	200	1.60	1.60	0.00
Mill soap base + 1.6% hexa.	None	0.00	200	1.60	1.60	0.00
Mill soap base + 1.8% hexa.	Amyl. sal.	Amyl salicylate 100	2.00	168	2.26	1.80	+0.46
Mill soap base + 1.8% hexa.	Amyl. sal.	Amyl salicylate 100	0.80	182	1.96	1.80	+0.16
Mill soap base + 1.8% hexa.	None	0.00	195	1.70	1.80	-0.10
Mill soap base + 2.0% hexa.	Vanillin	Vanillin 100	2.00	180	2.00	2.00	0.00
Mill soap base + 2.0% hexa.	None	0.00	180	2.00	2.00	0.00

100 ml. volumetric flasks. Into six of these add 5, 10, 15, 20, 25, and 30 ml. of standard hexachlorophene solution, respectively, leaving the seventh sample as a blank. These contain 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 0.0% hexachlorophene in a 5.0-gram soap sample. Add 55-60 ml. ethyl alcohol and two or three Hengar granules to each flask and heat to boiling point on a hot plate over an asbestos wire gauze until all of the soap is dissolved.* Remove the flasks singly and add 30 ± 1 ml. of barium bromide solution while the soap solution is still at the incipient boiling point.

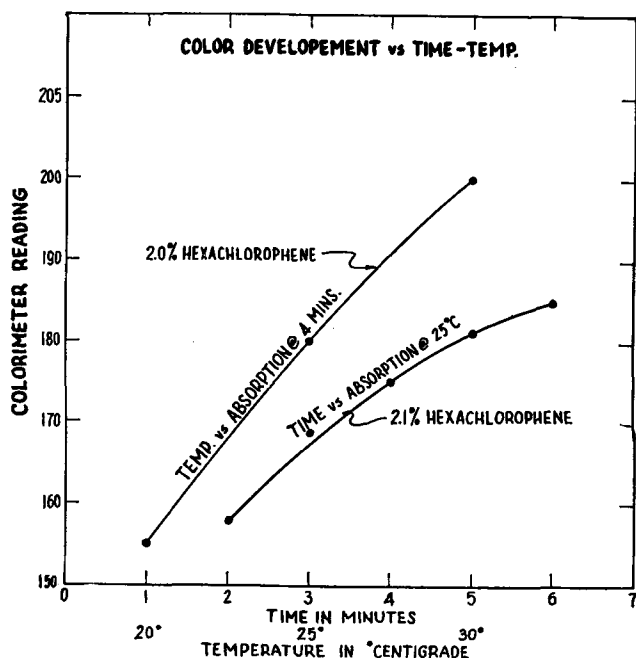


FIG. 1. Effect of time and temperature on colorimeter reading.

Swirl for about one minute or until the slurry is freed of gel-like agglomerations. Cool, adjust the volume to 100 ml. at $25 \pm 0.2^\circ\text{C}$., and then mix thoroughly.** Filter about one-half of the mixed slurry, collecting the filtrate in a 50-ml. volumetric flask. (A slight turbidity of barium carbonate from carbon dioxide in the air may develop in the filtrate upon standing, but this will disappear completely after dilution and the addition of ferric chloride.) Pipette a 5.00-ml. aliquot into a 50-ml. volumetric flask, add 15.00 ml. ethyl alcohol at $25 \pm 0.2^\circ\text{C}$., and then add 1.00 ml. of ferric chloride reagent while swirling the flask, allowing the pipette to drain for 25 seconds. Mix well. After reacting 3.3 min. at $25 \pm 0.2^\circ\text{C}$., fill an absorption cell and determine the light absorp-

tion in a photoelectric colorimeter without a filter, 4 min. \pm 10 ses. after the first of the ferric chloride has been added. With a spectrophotometer, or if a filter with the colorimeter is preferred, use a wavelength of $550 \text{ m}\mu$.

Plot the readings vs. hexachlorophene concentration to form the standard curve. It will be permanently valid on the same instrument and will be applicable to all milled sodium soaps.

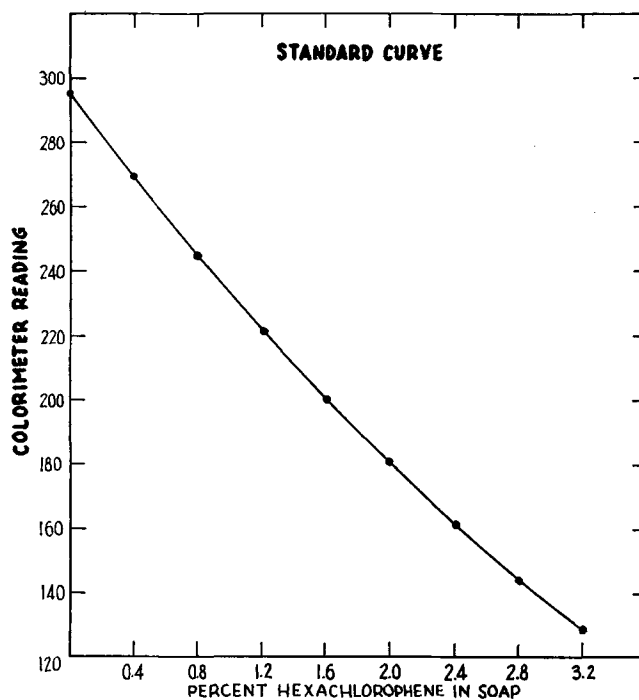


FIG. 2. Typical standard curve.

Determination

1. *For Milled Sodium Soaps.* Weigh 5.0 ± 0.05 grams of soap, containing less than 50% water, into a 100-ml. Pyrex brand glass volumetric flask. Proceed from here as in the preparation of the standard curve, starting with the fourth sentence (at single asterisk). Determine the percentage of hexachlorophene from the standard curve.

2. *For Liquid Potassium Soaps.* Weigh 5.0 ± 0.05 grams of liquid potassium soap into a 100-ml. Pyrex brand glass volumetric flask. Add 45 ml. of 95% ethyl alcohol, solubilize by swirling, without heating, and add 30 ml. barium bromide solution diluted to 50 ml. with ethyl alcohol. Proceed from here as in the preparation of the standard curve for sodium soap, starting with the seventh sentence (at double

asterisk). A separate standard curve should be made for liquid potassium soaps as potassium in place of sodium will shift the curve slightly so that somewhat lower results are obtained. An approximation can be made however by adding 0.10% to the result obtained from the sodium soap standard curve.

Calculations

Calculate the percentage of hexachlorophene present to any standard moisture content by determining the moisture on another portion of the original sample and using the expression:

$$\% \text{ actual hexachlorophene} = H - 0.06 G.$$

$$\% \text{ actual hexachlorophene @ standard H}_2\text{O content} = (H - 0.06 G) \times (100 - S) / 100 - A.$$

where

H = % hexachlorophene from standard curve.

G = % glycerol in the soap.

S = % standard H₂O.

A = % actual H₂O.

Notes

1. The absorption cell is cleaned by rinsing with alcohol and then acetone and air-dried. Any film remaining on the glass must be wiped away, using a clean cloth. The surfaces of the cell must be free of streaks and solvent residue before use.

2. Before use each time, the instrument is checked for balance with the clean and dry adsorption cell in place.

3. Not enough barium bromide will be present to form completely the barium soap from a sodium soap made exclusively with coconut oil fatty acids. In such an unlikely sample use only a 4-gram sample and compute the hexachlorophene content by dividing the result obtained from the sodium soap standard curve by 0.80.

4. In the preparation of the standard curves a soap with less than 1% of glycerol content should be used.

The barium soap precipitate must be well dispersed, otherwise a little occlusion of hexachlorophene will occur. With milled bar soaps a crystalline, well dispersed precipitate will always be obtained. A very high percentage of high iodine value acids in the fatty acid composition, such as from corn or soya oils, may give a conglutinated barium precipitate at the incipient boiling point, but milled sodium bar

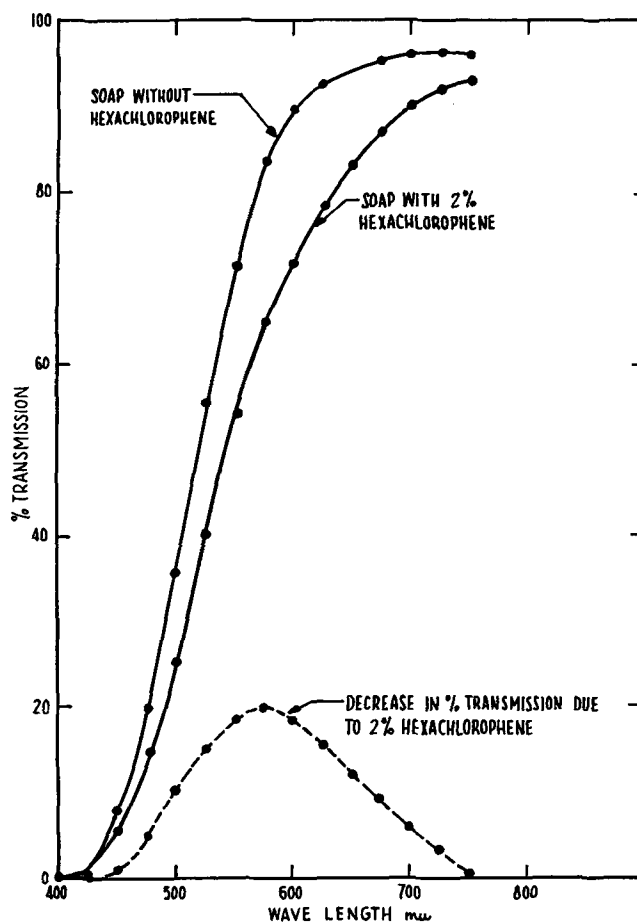


Fig. 3. Wave length-per cent transmission for the colored reaction solution. Scanning was started 15 minutes after the addition of the ferric chloride reagent so that very little fading would occur during the 18-20 minutes required for each figure. A Beckman DU Quartz Spectrophotometer was used.

soaps are not made with a high quantity of such acids so that this difficulty will not happen with these soaps.

Liquid potassium soaps however are invariably made from fatty acids containing a high percentage of high iodine value acids. With these soaps the precipitation is made at room temperature to prevent a conglutinated precipitate from forming and to assure a well dispersed precipitate. Alcoholic solutions of sodium soaps however gel at or near room temperature, necessitating the precipitation with these soaps at the incipient boiling point.

All of the sodium or potassium bromide formed, together with the excess BaBr₂ reagent, is soluble in the alcohol solution. The soluble bromides contribute to the optical density of the final colored solution. In the method the same quantity of barium bromide reagent is always used, giving the same bromide concentration each time and eliminating it as a variable. A one-milliliter variation of BaBr₂ reagent makes a 0.024% hexachlorophene content variation in the result.

A 5-gram sample of soda soap containing between 100% and down to at least 50% of anhydrous soap does not contribute any inherent error to the result, allowing a corresponding wide variation in moisture content of the sample.

TABLE III
Typical Analyses

No.	Soap (gm.)	Hexa. (gm.)	Total wt. (gm.)	Actual hexa. in sample (recrystallized) %	Hexa. found in sample, %	Error, %
1	5.004	0.1005	5.105	1.97	2.02	+0.05
2	5.005	0.0870	5.092	1.71	1.77	+0.06
3	5.113	0.1390	5.252	2.64	2.57	-0.07
4	4.999	0.0752	5.075	1.48	1.52	+0.04
5	5.049	0.1058	5.154	2.05	2.13	+0.08
6	5.005	0.1320	5.137	2.57	2.59	+0.02
7	3000 lb. @ 30% H ₂ O	55 lb. other ad- ditives)	2437 lb. @ 11.5% H ₂ O	2.25	2.19	-0.06
8	5.000	0.1053	5.105	2.06	2.00	-0.06
9	5.000	0.0745	5.074	1.47	1.48	+0.01
10	3000 lb. @ 30% H ₂ O	48 lb. other ad- ditives)	2430 lb. @ 11.5% H ₂ O	1.97	1.88	-0.09
11	4.93 ^a	0.0707	5.00	1.42	1.41	-0.01
12	4.91 ^a	0.0960	5.00	1.97	1.89	-0.08
13	4.92 ^b	0.0750	5.00	1.50	1.45	-0.05
14	4.87 ^c	0.1250	5.00	2.50	2.45	-0.05

^a These samples are actual mill soap bars containing perfume, color, pigment, and preservative. They were prepared in such a manner, so that the hexa. could be precisely determined by calculation.

^b Commercial product. Contains ca 96% hexa.

^c Liq. soya-cocoa potassium soap, 34% conc. No glyc.

While this method was developed and used exclusively with milled sodium soaps, the method can be used with liquid potassium soaps if it is modified slightly. At room temperature, barium bromide precipitates diluted liquid potassium soaps of any fatty acid composition in a finely dispersed form. With liquid potassium soaps, as with milled sodium soaps, a wide variation in the anhydrous soap content of the sample does not introduce any inherent error in the method; consequently the moisture content of the sample does not introduce any error. As these soaps are likely to contain several per cent of glycerol, its content in the soap should be known and corrected, as shown in the expression for the calculations of hexachlorophene.

Potassium soaps will give slightly less light absorption than sodium soaps with this method so that the standard curve prepared from sodium soap will not be directly applicable to potassium soaps, and a separate standard curve should be prepared.

These soaps frequently contain an organic divalent sequestrant, ethylene diamine tetra acetic acid, but it will not introduce any error in the result even if up to 0.70% of the dry material is present. The trivalent sequestrant, nitritotriacetic acid, however, will give an error of +0.02% hexachlorophene for each 0.047% of dry material present in the soap. These are about the usual quantities of the two sequestrants used in liquid potassium soaps.

Summary

Hexachlorophene is determined in soap by measuring the color produced by reaction with ferric chloride in alcohol solution with controlled conditions of time and temperature. The precision is within $\pm 5\%$ of the hexachlorophene content at the $\frac{1}{2}\%$ level or above. As a criterion, any soap that will give a finely dispersed barium soap and that does not form a precipitate with ferric chloride under the conditions of the method give good results. This includes all milled bar soaps. The effect of phenolic perfume ingredients on the result is negligible. Five per cent abietic acid in the fatty acid composition has no effect on the result. The method is slightly modified for use with liquid potassium soaps.

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The Chronic Toxicity of Lauryl Gallate¹

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IN the quest for satisfactory antioxidants that might be used to retard development of rancidity in fats, oils, and similar materials, the higher fatty alcohol esters of gallic acid have shown solubility advantages over such powerful antioxidants as gallic acid and nordihydroguaiaretic acid. The antioxidant properties and advantages of octyl, dodecyl, tetradecyl, hexadecyl, and octadecyl gallates have been reported by Morris, Kraekel, Hammer, Myers, and Riemenschneider (1), and the direct esterification of gallic acid with higher alcohols has been described by Ault, Weil, Nutting, and Cowan (2).

Lauryl (dodecyl) gallate is typical of the above-mentioned fatty alcohol esters of gallic acid in regard to antioxidant effectiveness in shortening and in retarding developments of rancidity in baking goods as judged by pie-crust tests. The use of lauryl gallate in edible substances requires data on the acute and chronic toxicity to permit evaluation of any undesirable effects that might result from the ingestion of small amounts of the compound at frequent intervals over a long period of time.

In discussing "Some Factors Affecting the Control of Oxidative Rancidity," Hilditch (3) stated that "the

possible toxicity of ethyl gallate has been investigated by Prof. J. A. Gunn of Oxford, at the request of the Medical Research Council and by Prof. A. D. Macdonald of Manchester. Both reported that no symptoms of toxicity were observed in mice, which had received orally or subcutaneously massive doses of ethyl gallate, in concentrations far greater than could ever be approached by human beings when receiving foods stabilized against oxidation by the ester." This statement is not explicit as to whether the tests on mice were of an acute or chronic nature. In view of the use proposed for the gallic acid esters, data on chronic toxicity based on long term feeding experiments would be the more important.

If it is assumed that lauryl gallate is hydrolyzed in the digestive tract, there is little reason to believe that the resulting lauryl alcohol and gallic acid would exert any toxic actions. Lauryl alcohol might be oxidized to lauric acid, which occurs as a glyceride in many vegetable fats, for example coconut oil. Gallic acid is widely distributed in the form of gallo-tannin in vegetable foods. Gallo-tannin is not absorbed from the gastro-intestinal tract but is hydrolyzed to gallic acid, which is readily absorbed and oxidized in the body. Although such speculation suggests that the repeated daily ingestion of reasonable amounts of lauryl gallate would not produce chronic toxicity,

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