An AutoAnalyzer Method for Determining Hexachlorophene in Soap

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Abstract

An AutoAnalyzer method has been developed for determining hexachlorophene in soap. The free phenol group is condensed with 4-aminoantipyrine, and after oxidizing with alkaline potassium ferricyanide, the resulting quinone-type dye is colorimetrically measured at 480 mμ. Internal standards are employed having known amounts of hexachlorophene. The method is applicable to the range that would be expected in a germicide soap, i.e. from 0–2%. The overall precision was found to be ±0.022% (95% confidence limits) at the 1.00% level of hexachlorophene in soap. The presence of other germicide types such as for example, carbanilides or salicylanilides, should not interfere with the determination. This routinized method should prove useful in operations where numerous repetitive measurements are made.

Introduction

THE TECHNICON AUTOANALYZER automates a variety of manipulations or steps in colorimetric analysis (1). Such automated steps include sampling, dilution, reagent addition, mixing, and measuring and recording of optical densities. The AutoAnalyzer is found particularly useful in applications where numerous routine or repetitive analyses are required. Typical examples are continuous monitoring of blood and other body fluids, and use in chemical process systems (2). Recent applications have been made in areas of interest to the detergent industry, for example in phosphate analysis (3) and in biodegradability studies where ABS or LAS contents of river water and sewage effluents were continuously measured (4,5).

In this paper we would like to report on the extension of the AutoAnalyzer to analysis of germicide, specifically hexachlorophene, in bar soaps. The method was developed as a means for monitoring processed soap and accordingly to increase the quality control capability of a soap manufacturing facility.

Spectrophotometric procedures of fairly wide applicability for germicides were recently furnished by Jungermann and Beck (6). Hexachlorophene was extracted from soap with dimethylformamide then determined by UV absorption at roughly 300 mμ. Other workers have employed colorimetric procedures useful for phenols, such as measurement of a ferric chloride complex (7) or condensation with 4-aminoantipyrine in the presence of alkaline oxidizing agents to yield a reddish antipyrine dye (8–10). We have found the method involving the 4-aminoantipyrine reagent as most suitable for adapting to the AutoAnalyzer.

Experimental

Equipment

1) AutoAnalyzer (Technicon Controls, Inc.): *a,* sampler II; *b,* proportioning pump (2-speed); *c,* colorimeter—equipped with 15 mm flow cell, 480 mμ filter and No. 1 aperture, model 1; *d,* recorder (single pen); *e,* pump and transmission tubing, connectors and coils.

2) Regular laboratory equipment as required.

Reagents

1) 2% solution of 4-aminoantipyrine in distilled water.

2) 8% solution of potassium ferricyanide in distilled water.

3) 0.10% of sodium carbonate in distilled water.

4) 50% alcohol (SDA 3A diluted with distilled water).

Procedure

1) AutoAnalyzer Setup (Fig. 1)

The 4-aminoantipyrine solution is pumped at 0.42 cc/min and immediately joined by sodium carbonate solution at 3.90 cc/min. The sample tube pumps at 3.39 and the air at 0.8 cc/min. Sample, air and the diluted 4-aminoantipyrine solution are then joined into one stream by means of a G-2 cactus connector and passed through a small mixing coil (Technicon No. 105–88). The stream from the coil is then further diluted by introducing more sodium carbonate solution at 3.90 cc/min. After passing through another small coil, the system is joined by the potassium ferricyanide solution pumping at 0.32 cc/min. The resulting solution is passed through two longer mixing coils packed with small beads (Technicon No. 116–101–2), another small coil and then to a separator (B-4) module. From the separator, a portion of the stream is pumped, at 3.39 cc/min, to the colorimeter. Another tube pumps from the colorimeter flow cell to waste at 2.89 cc/min.

2) Preparation of Samples:

a) *Standards:* Prepare 0.5% solutions of soap, known to contain no hexachlorophene, in 50% alcohol.

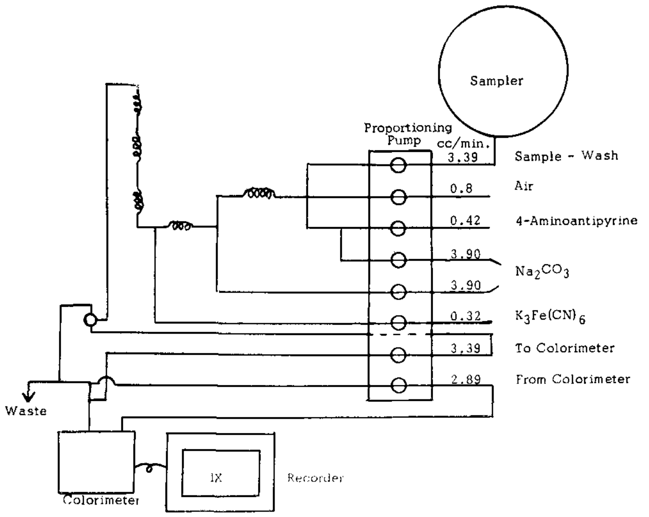


FIG. 1. Schematic of AutoAnalyzer set-up.

Include various amounts of hexachlorophene from 0.00% to 1.00%. Filter the solutions if they are not clear.

b) *Unknowns for Analysis:* Prepare 0.5% solutions in 50% alcohol and filter if not clear. Should the sample prove to contain more than 1% of hexachlorophene, a suitable dilution should be made.

3) Hexachlorophene Determination

a) Start the AutoAnalyzer pumping the reagents, drawing 50% alcohol through the sample tube, and run until a steady baseline is established on the recorder chart. Adjust the baseline to 0 (100% transmission).

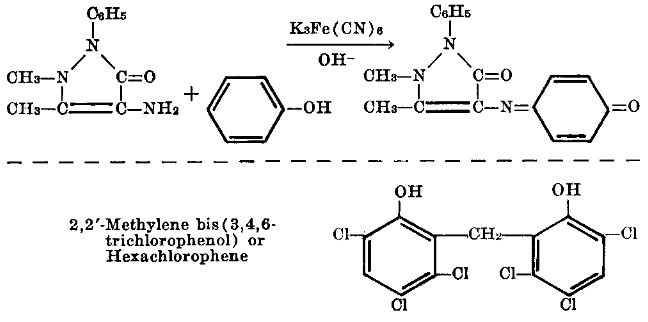
b) Start sampler motor, sampler being loaded with alternate samples (or standards) and washes (50% alcohol) and operated at a speed of 50 samples and/or washes per hour. In place of every fifth sample, insert a standard (soap solution of known hexachlorophene content).

c) Calculations:

$$\% \text{ Hexachlorophene} = \frac{\% \text{ in standard} \times \text{sample optical density}}{\text{Optical density of standard}}$$

Results and Discussion

The reaction as described by Emerson (8) for phenol is as shown in Scheme I. 4-Aminoantipyrine is considered to react through its amino group with phenolic compounds. The reaction is conducted in the presence of alkali and the resulting condensate is oxidized to a reddish quinone compound by potassium ferricyanide. It was concluded that substitution took place at the position para to the phenolic —OH group. The phenolic could originally be unsubstitued, or have halogen, carboxyl, sulfonic acid, hydroxyl or methoxy groups in the para position. These groups apaprently were expelled in the reaction. If para substituents were alkyl, aryl, nitro, benzoyl or aldehyde groups, reaction did not occur. Certain exceptions to these rules have been observed and, because there is still some uncertainty, the reaction product with hexachlorophene is not explicitly shown.



SCHEME I. Condensation of 4-aminoantipyrine with phenolics.

Fig. 2 gives absorbancy versus wavelength curves for reaction products of various hexachlorophene extracts with 4-aminoantipyrine. These hexachlorophene extracts were derived from four differently colored soaps, namely white, aqua, pink and gold as derived from combinations of various dyes. While the extracts were highly colored depending on the soap dye employed, dilution in the procedure to 0.14% soap is sufficient to overcome this interference.

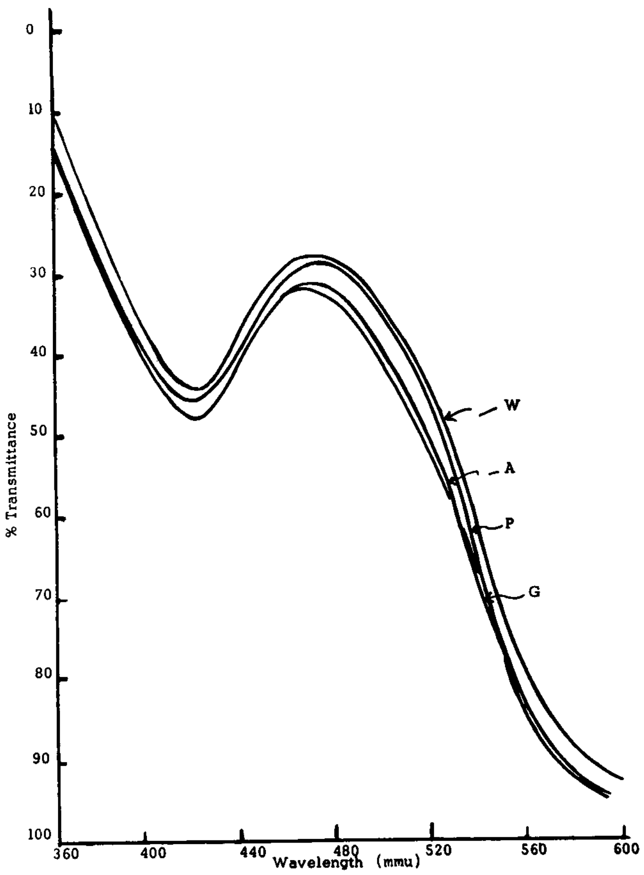


FIG. 2. Visible spectra for soap containing 0.83% hexachlorophene (Beckman DB Sargent Recorder).

Maximum absorbancy occurred in the region of 470 to 480 mμ. In our own work the wavelength of 480 mμ was employed throughout. It should also be noted that in the procedure, use of the AutoAnalyzer requires that internal standards be run along with samples. This calibrates the instrument and compensates for minor shifts in optical densities. Internal standards were run in this procedure and these were obtained by adding known quantities of hexachlorophene to soap solutions.

Results for a series of known hexachlorophene levels are given in Fig. 3. This is a reproduction of a trace

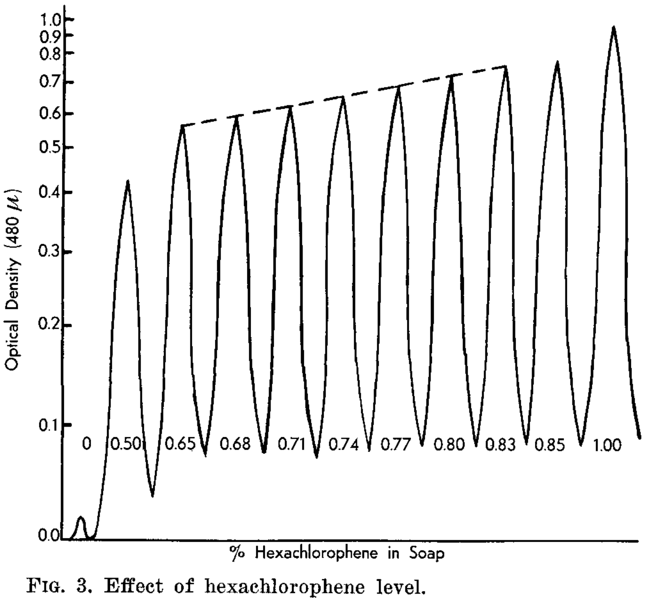


FIG. 3. Effect of hexachlorophene level.

TABLE I

Precision of Hexachlorophene Values Related to Sampling Rate

Actual hexachlorophene content (%)	% Hexachlorophene 50 rotations/hr.	determined at: 70 rotations/hr.
0.72	0.72	0.70
0.72	0.72	0.73
0.72	0.72	0.72
0.72	0.72	
0.72	0.72	
0.75	0.74	0.74
0.75	0.76	0.78
0.75	0.76	0.73
0.75	0.75	
0.75	0.74	
95% C.L.	±0.02	±0.08

as it appeared on the recorder chart. It is noted that there is a small blank. However, the blank has no significance since internal standards serve to cancel it out. Measurements are made by noting the distance from the baseline to the peak height. The fact that the curves do not return to the base line is of no consequence; again, because internal standards are included. This chart also shows that observed optical densities are linearly related to the hexachlorophene level. A linear response is of course important for providing precise results.

Precision of data is illustrated in Table I. Data are given for two operating speeds in sampling: 50 rotations per hour and 70 rotations per hour. It is apparent that reproducibility diminishes at the higher sampling rate where a steady state in reaction or colorimeter response is not achieved. For this reason the 50 rotation per hour operating rate was selected and this is equivalent to 20 samples per hour. At this sampling rate precision at the 95% confidence level is ±0.02 units of percent hexachlorophene in soap. This variation was found essentially constant in the range of hexachlorophene from 0.5 to 1%.

TABLE II

Accuracy (% Recovery of Hexachlorophene)

Soap color	Samples tested	% Recovery mean	Variance	Error of mean[a]
White	40	99.3	5.72	0.76
Gold	35	100.4	3.71	0.66
Pink	34	100.4	1.35	0.41
Aqua	35	100.0	1.02	0.35
Pooled	144	100.0	3.06	0.29

[a] 95% *Confidence* level.

Accuracy of the method is demonstrated by results in Table II. Here, percent recoveries are given for four differently colored soap bars. In these studies, data were obtained for soaps ranging from 0.5 to 1.0% in hexachlorophene content. Recoveries ranged from 99.3 to 100.4% and were found to be independent of bar color and hexachlorophene content at the 95% confidence level. On pooling the data, an overall accuracy of 100 ± 0.2% is obtained.

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