Colorimetric Determination of Chlorobutanol in Injections *Via* the Fujiwara Reaction

By LESTER CHAFETZ* and ROBERT W. MAHONEY

A colorimetric assay based on the pink color produced in the Fujiwara alkali–pyridine reaction is described for chlorobutanol in parenteral dosage forms. Accuracy is adequate, and the procedure is rapid and selective.

THE OFFICIAL assay of chlorobutanol (1), chloride titration after hydrolysis, is not suitable for determination of the compound in injections. The method has been modified using an amperometric end point (2) to obtain sufficient sensitivity. Other methods reported for the preservative in pharmaceuticals include polarography (3), a biological assay using a turbidimetric end point (4), and a colorimetric method based on the ferric hydroxamate complex (5), which is subject to interference by esters.

A colorimetric procedure based on the Fujiwara alkali–pyridine reaction for chloroform (6), which is suitable for determination of the chlorobutanol in unit-doses of injections, is described here. Application of the reaction to various polyhalogen compounds was reviewed by Truhaut (7), who reported that chlorobutanol gives an intense color in the Fujiwara reaction, but supplied no analytical procedure.

EXPERIMENTAL

Equipment and Supplies.—Chlorobutanol U.S.P. (Merck No. 20721), thiophene-free benzene, reagent grade pyridine, sodium hydroxide, a filter photometer (Leitz Photrometer), a clinical centrifuge, and conventional laboratory glassware were used in the study. Quantities given are in terms of anhydrous chlorobutanol.

Procedure.—Dilute the sample solution to contain about 100 mcg./ml. of chlorobutanol. Shake 5.0 ml. of the dilution with 5.0 ml. of thiophene-free benzene. Clarify the benzene layer by centrifuging and transfer 1.0 ml. to an 18 × 150 mm. test tube. Add 2.0 ml. of 20% sodium hydroxide and mix. Add 10.0 ml. of pyridine, mix, and heat the tube in a boiling water bath for 4 min. Allow the mixture to cool for 5 min., then transfer 5.0 ml. of the pyridine layer to a tube containing 1.0 ml. of distilled water. Mix, and determine the absorbance of the pink solution at 535 mμ in a suitable colorimeter within 15 min. Compare the absorbance of the sample, A_u, with the absorbance of a concomitantly run standard, A_s, freshly prepared from U.S.P. chlorobutanol. Distilled water is suitable for setting the colorimeter to null.

$$\text{chlorobutanol, mg./ml.} = A_u/A_s \times \text{mg. in standard} \times \text{dilutions}$$

Effect of Temperature on Reaction.—Truhaut (7) suggests that 60° is optimum for determination of chloroform. Any possible gain in sensitivity or stability by thermostating the reaction mixture was believed to be compensated by the convenience of using a boiling water bath, so the effect of temperature as a variable was not evaluated.

Effect of Alkali Concentration.—The general procedure was followed using concentrations of sodium hydroxide ranging from 10 to 40%, starting with a chlorobutanol standard concentration of 114 mcg./ml. The results are presented in Table I.

Effect of Heating Time.—The effect of time in the boiling water bath was evaluated using a stock

TABLE I.—EFFECT OF ALKALI CONCENTRATION

% NaOH	10	20	30	40
$A_{535\ m\mu}$	0.321	0.398	0.286	0.032

TABLE II.—EFFECT OF HEATING TIME

Min. in Bath	1	3	5	7
$A_{535\ m\mu}$	0.317	0.432	0.427	0.394

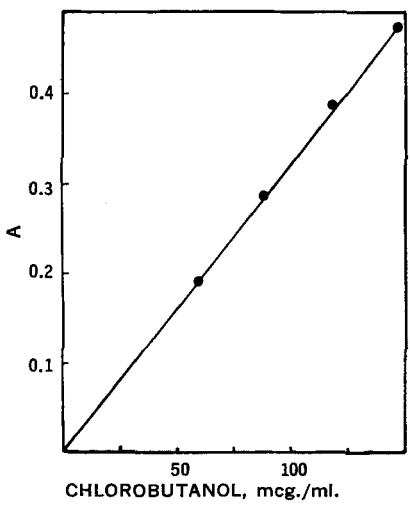

Fig. 1.—Standard curve for chlorobutanol in the Fujiwara reaction using the 535 mμ filter in the Leitz Photrometer.

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TABLE III.—ASSAY OF COMMERCIAL INJECTIONS

Product	pH	Chlorobutanol Claim, %	Chlorobutanol Found, %	Chlorobutanol % Recovery of Added[a]
Thiamine HCl, 100 mg./ml.	2.61	0.35	0.27	96.0
Isoniazid, 100 mg./ml.	6.01	0.50	0.47	98.3
Oxytocin, 10 units/ml.	3.52	0.50	0.56	...

[a] See text.

dilution of 132 mcg./ml. The results are presented in Table II.

Adherence to Beer's Law.—Beer's law was followed strictly in the concentration range studied. A typical Beer's law plot is presented in Fig. 1, where each point is the average of at least duplicate samples.

Assay of Chlorobutanol in Commercial Injections.—The standard procedure described above was used to estimate chlorobutanol in thiamine hydrochloride, isoniazid, and oxytocin vials. These were products of indeterminate age from hospital stocks. There was considerable variance noted in the declared and found concentrations of preservative in the preparations as shown in Table III. The assays were validated by recovery experiments in which known amounts of preservative were added to two of the commercial preparations. The amount recovered was calculated from assay values with and without added chlorobutanol. All data represent the average of duplicate or triplicate assays.

SUMMARY

A procedure for estimating chlorobutanol in parenteral dosage forms is described. The procedure requires only commonly available laboratory equipment, and it is rapid and selective. The sensitivity is sufficient for use in assay of unit doses. The method appears to be accurate to about ±5%.

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Infrared Identification of Indole Ring in Indoles and Indole Alkaloids in the 700 to 400 cm.$^{-1}$ Region

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The spectra of 23 indoles of varying structure have been recorded and analyzed in the range of 700 to 400 cm.$^{-1}$. All of the indoles studied have two characteristic absorption bands in this region. The first is located at 620 ± 20 cm.$^{-1}$ and the second at 575 ± 25 cm.$^{-1}$. Spectra-structure correlations of the absorption bands are presented and discussed. These bands appear to be characteristic of the indole moiety and should be useful for verification of suspected indole structures.

THE AVAILABILITY of commercial instrumentation extending into the far infrared region has heightened interest in this relatively unexplored area. As various functional groups and classes of compounds are investigated, the region gains in importance. Previous publications have shown that certain functional groups and ring systems have characteristic absorptions in this area. Aldehydes (1), ketones (2), amides (3), and aliphatic and aromatic hydrocarbons (4) are among those shown to possess unique absorptions in the far infrared. With few exceptions, however, there has been little reported on heterocyclic systems. This paper reports the results of an infrared study of the biologically important indole ring system. Twenty-three indoles of varying structure have been studied in the 700 to 400 cm.$^{-1}$ region, and all show two characteristic bands in this range. This study provides definitive information on the presence of the indole ring, a feature which is not available in the 4000 to 650-cm.$^{-1}$ region.

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EXPERIMENTAL

With the exception of 4-methoxyindole, which was synthesized at these laboratories, all of the indoles studied were commercially available.

The spectra were recorded on a Perkin-Elmer model 521 spectrometer. The samples were prepared and run as mineral oil mulls on KBr plates in the case of solids and as natural films in the case of liquids.

RESULTS AND DISCUSSION

Table I lists the indoles studied and the major absorption bands found to be characteristic for the series. It can be readily seen that the range of correlation frequencies is a narrow one and varies by ±20 cm.$^{-1}$ for the first absorption and ±25 cm.$^{-1}$