A NEW COLORIMETRIC METHOD FOR THE DETERMINATION OF CHLORAL HYDRATE

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A colorimetric method is described for the determination of chloral hydrate, particularly in the presence of its decomposition products. Quinaldine ethiodide reacts with chloral hydrate in alkaline solution to produce a stable, blue cyanine dye. Chloroform, trichloracetic acid and formic acid do not react under the conditions described. The colour produced conforms to the Beer-Lambert law up to 100 μg./10 ml. of reaction mixture.

In the course of work on the stability of chloral hydrate preparations it became necessary to determine chloral hydrate in the presence of its decomposition products, viz. trichloracetic acid, formic acid, hydrochloric acid[1] and possibly chloroform. Conventional macro methods for estimating chloral hydrate, based on alkaline hydrolysis or total chlorine estimation, are subject to interference from the decomposition products mentioned above and in an attempt to find a more specific method, attention was turned to colorimetric methods.

With one exception, colorimetric methods for the estimation of chloral hydrate[2–7] are based on the reaction of chloral hydrate and pyridine in alkaline solution to produce a red colour, Fujiwara's reaction[8] (also attributed to Ross[9]). Although sensitive, this is not specific for chloral hydrate and a positive reaction is given by many poly-halogenated aliphatic compounds, including chloroform and trichloracetic acid. As a result, chloral hydrate in the presence of trichloracetic acid must be determined by difference. Meyer and Lee-Motter[5] determine both compounds together by means of the Fujiwara reaction and trichloracetic acid after hydrolysis of the chloral hydrate and removal of the chloroform produced; Friedman and Cooper[7] use a similar procedure but make use of the maximum at 370 mμ instead of that at 540 mμ. Fujiwara's reaction has been used for the colorimetric estimation or detection of many polyhalogen compounds; trichloroethylene[10,11], trichlorethanol[5,7,10] carbon tetrachloride[12], urochloralic acid[5], chloroform[13,14], chloralose[15] trichloracetic acid[5,7,10,16], tertiary acetylenic halogenated alcohols[17] and chloral urethane[18].

Stehwien and Kühmstedt[19] describe a colour reaction of chloral hydrate, which they adapted for quantitative purposes, based on the reaction of chloral hydrate, hydroxylamine hydrochloride and 2,6-diaminopyridine in acid solution to produce a red pyrisatin dye. Although apparently specific, the reaction conditions are critical and the reaction is relatively insensitive. In addition 2-6-diaminopyridine is not readily available.

Other colour reactions of chloral hydrate described in the literature[20–23] also lack selectivity in that similar colours are produced by other aldehydes or halogen compounds.

The colour reaction of Feigl[24], although specific, has the disadvantage of using concentrated sulphuric acid, which produces colours or charring with many organic compounds.

In 1934 Ogata and Suzuki[25] reported the preparation of cyanine dyes from chloral hydrate by condensation in alkaline solution with quinaldine

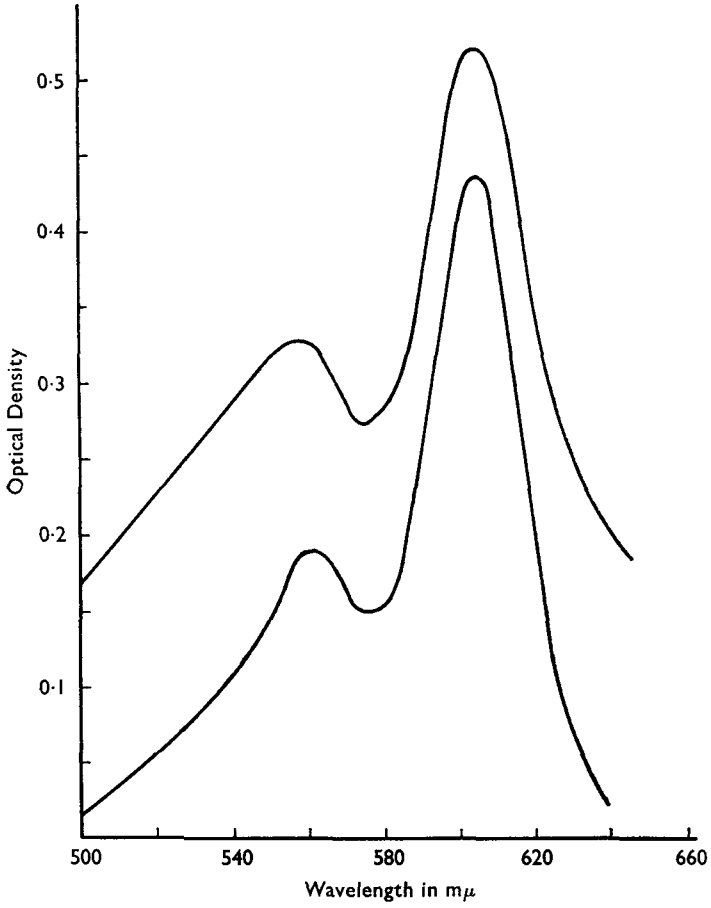


FIG. 1. Comparison of absorption spectra.
Upper curve: colour obtained from chloral hydrate.
Lower curve: 1,1′-Diethyl-2,2′-carbocyanine iodide.

ethiodide, lepidine ethiodide and α-picoline ethiodide. This reaction was examined with a view to adapting it to the quantitative colorimetric estimation of chloral hydrate.

EXPERIMENTAL

Preliminary experiments showed that quinaldine ethiodide was the most sensitive reagent and was studied in detail; chloroform and trichloracetic

acid were found not to react and the reaction was therefore investigated further.

Ammonia and (mono)ethanolamine were found to be the most effective alkalies and the presence of a water soluble alcohol increased the sensitivity of the reaction. The colour produced has a sharp maximum at 605 mμ and a wider maximum at 555–560 mμ (Fig. 1). The maximum at 605 mμ was used in subsequent quantitative measurements. The following variables were studied in more detail to determine the optimum conditions (all experiments were carried out in test-tubes graduated at 10 ml. with a final volume of reaction mixture of 10 ml.).

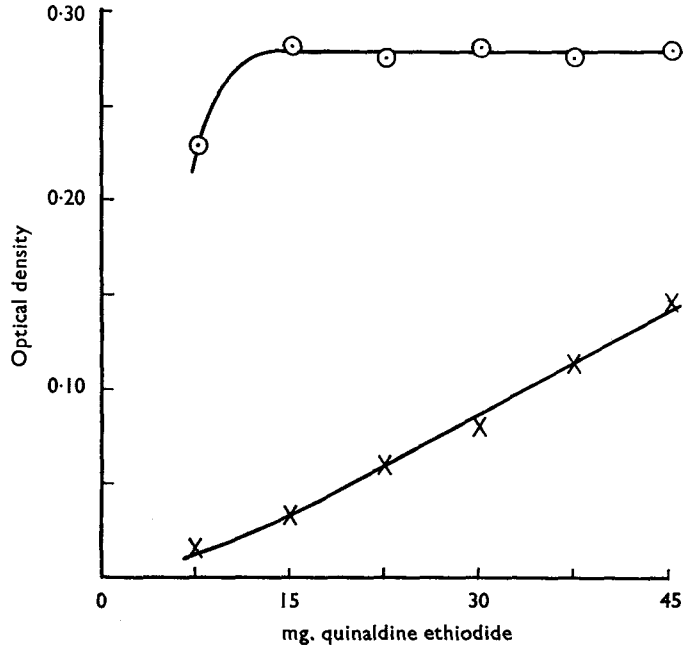


FIG. 2. Effect of varying quantities of quinaldine ethiodide.

⊙—⊙ Optical density at 605 mμ; 50 μg. chloral hydrate measured against appropriate blank.

×—× Optical density at 560 mμ; blank measured against water.

Alkali. Ammonia and ethanolamine were found to be the most effective alkalies and the less volatile ethanolamine was studied in detail. The colour produced is constant for 0·2 to 0·8 ml. of 0·1N ethanolamine in 10 ml. of reaction mixture.

Quinaldine ethiodide. The effect of varying amounts of quinaldine ethiodide on the colour produced from 50 μg. of chloral hydrate is shown in Figure 2. The colour produced is constant with 15 mg. or more in 10 ml. of reaction mixture but with increasing amounts the faint pink colour of the blank (maximum at 560 mμ) increases as shown in Figure 2 and the blue colour due to the chloral hydrate assumes a reddish tint.

Alcohol. Isopropanol and n-propanol were found to be effective in improving sensitivity; isopropanol was used throughout. Maximum colour is produced with 6 or more ml. of isopropanol in the reaction mixture.

Time and temperature. The reaction is slow at temperatures of 50° or below, but the rate of reaction increases at higher temperatures. 60° was chosen as a convenient working temperature. The colour reaches a maximum after 50 minutes at 60° and is constant for a further 40 minutes heating.

Chloral hydrate. Using the conditions described in the method the colour produced was proportional to the amount of chloral hydrate present up to 100 μg. in 10 ml. of reaction mixture. The colour produced is stable for at least 24 hours.

Method

Reagents. Quinaldine ethiodide solution 1·5 per cent w/v: dissolve 1·5 g. quinaldine ethiodide in water and dilute to 100 ml. and filter if necessary. 0·1N Ethanolamine: dissolve 6·1 g. ethanolamine B.P. in water and dilute to 1 litre. Isopropanol: analytical reagent grade. Standard chloral hydrate solution (50 μg./ml.): Dissolve 0·2500 g. chloral hydrate B.P. in water and dilute to 500 ml.; dilute 10 ml. of this solution to 100 ml. with water.

Apparatus. Test-tubes graduated at 10 ml. Unicam SP.500 spectrophotometer.

Procedure. Prepare an aqueous solution or extract of the sample to contain about 50 μg. chloral hydrate in 1 ml. Pipette 1 ml. of this solution into a graduated test-tube, pipette 1 ml. of standard chloral hydrate solution into a second graduated test-tube and pipette 1 ml. of water into a third graduated test-tube to serve as a blank. Add to each test-tube 1 ml. of quinaldine ethiodide solution and 6 ml. of isopropanol, mix and add 0·5 ml. of 0·1N ethanolamine; dilute to 10 ml. with water, mix, and place in a water bath at 60° for 1 hour. Remove from the water bath, cool, and measure the optical densities of the sample and standard against the blank at 605 mμ.

Calculate the amount of chloral hydrate present from the ratio:

$$\frac{\text{Optical density of sample}}{\text{Optical density of standard}}$$

If necessary prepare a solution or extract of the sample with isopropanol and take a suitable aliquot. Adjust the amount of isopropanol added later accordingly.

RESULTS

Some results obtained by the method are shown in Table I together with the results obtained by the B.P. or B.P.C. method, where applicable. The B.P.C. preparations were freshly prepared from accurately weighed quantities of chloral hydrate and diluted to volume in 100 ml. volumetric flasks.

The accuracy of the method is comparable with that of the appropriate B.P. or B.P.C. method.

Specificity of the reaction. 5 mg. quantities of the following substances (i.e., 100 times the quantity of chloral hydrate normally taken) were tested under the conditions described: trichloracetic acid, acetic acid, formic acid, oxalic acid, citric acid (added as equivalent amounts of sodium salts) chloroform, bromoform, hexachlorethane, pentachlorethane, chlorbutol, formaldehyde, acetaldehyde, benzaldehyde and glucose. In each case no blue colour was produced.

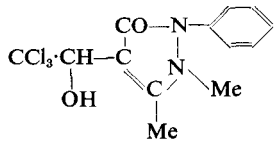
TABLE I

COMPARISON OF RESULTS FROM THE PROPOSED METHOD OF ESTIMATING CHLORAL HYDRATE WITH THOSE OBTAINED USING THE OFFICAL METHOD

	Chloral hydrate content per cent w/v		
	Calculated	Found	
		B.P. or B.P.C. method	Proposed colorimetric method
Syrup of Chloral B.P.C.	20·0	19·8	20·4
Mixture of Potassium Bromide and Chloral B.P.C.	2·29	2·30	2·32
Mixture of Chloral B.P.C.	9·14	9·11	9·10
Mixture of Chloral and Potassium Bromide for Infants B.P.C.	3·33	—	3·30
2-Methyl-2-hydroxy-4-(βββ-trichloro-α-hydroxyethoxy) pentane $CCl_3{\cdot}CH(OH){\cdot}CH(Me){\cdot}CH_2{\cdot}C(Me)_2{\cdot}OH$	62·3*	62·0*	63·0*
Dichloral phenazone $C_{11}H_{12}N_2O{\cdot}2\,CCl_3CH(OH)_2$	63·7*	63·6*	63·5*
Dichloral phenazone tablets	413**	409**	414**
Proprietary Product A containing chloral glycerolate	5·85	—	5·85
Proprietary Product B containing chloral hydrate, valerian and strontium bromide	2·11	—	2·18
Proprietary Product C containing chloral hydrate, sodium bromide and hyoscine hydrobromide	18·25	—	18·27

* per cent w/w. ** mg./tablet.

The related compounds, chloral formamide and butyl chloral hydrate, produced a green and reddish-violet colour respectively. Chloralose and the condensation product of chloral hydrate and phenazone, 4(βββ-trichloro-α-hydroxyethyl)-2,3-dimethyl-1-phenyl-pyrazol-5-one (I) produced no colour; the hemi-acetal with hexylene glycol, 2-methyl-2-hydroxy-4-(βββ-trichloro-α-hydroxy-ethoxy)-pentane (II) and the addition compound, dichloral phenazone, produced a blue colour in proportion to their chloral hydrate content.



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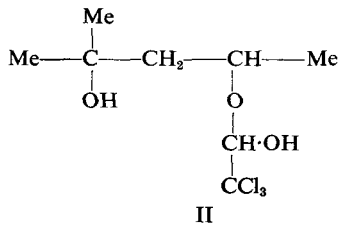
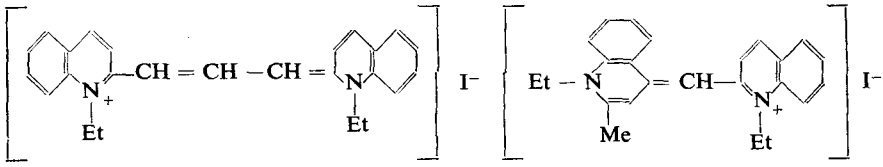


II

Discussion

Paper chromatography of the blue colour extracted from the reaction mixture showed it to be identical in chromatographic properties, in neutral, acid and alkaline solvents, with a sample of 1,1′-diethyl-2,2′-carbocyanine iodide, III, prepared by the method of Hamer[26]. The absorption spectrum of (III) is shown in Figure 1.

The faint pink colour of the blank, which increased with increasing quantities of quinaldine ethiodide, as shown in Figure 2, is believed to be due to the formation of a red isocyanine dye (IV), produced by the condensation of two molecules of quinaldine ethiodide.

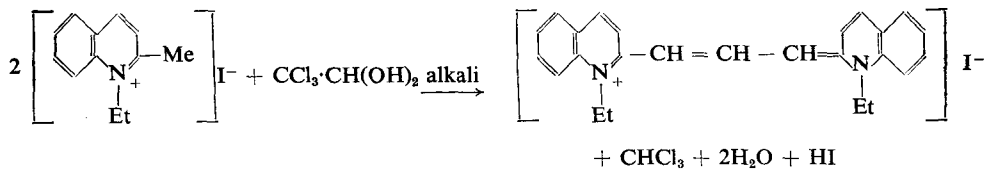


III
1,1′-diethyl-2,2′-carbocyanine iodide

IV
1,1′-diethyl-2′-methyl-2,4′-isocyanine iodide

(III) has been synthesised from quinaldine ethiodide by condensation with formaldehyde[27], chloroform[28] or ethyl orthoformate[26] as a source of the central carbon atom. Under the conditions described under the method, these compounds do not react.

Ogata and Suzuki formulated the reaction as involving the CCl_3 group of chloral hydrate, but the failure of compounds such as trichloracetic acid, chloral formamide and chloroform to produce a blue cyanine dye under the above conditions suggests that the $CH(OH)_2$ group is involved, with the elimination of one molecule of chloroform, thus:



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