THE DETERMINATION OF SMALL QUANTITIES OF CHLORHEXIDINE IN PHARMACEUTICAL PREPARATIONS

BY A. HOLBROOK

From the Department of Pharmacy, Imperial Chemical Industries Limited, Pharmaceuticals Division, Hexagon House, Blackley, Manchester, 9.

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A colorimetric method has been developed for the determination of small quantities of chlorhexidine by reaction in alkaline solution with sodium hypobromite. The precipitation of the base is prevented by the use of a surface-active agent. The method is rapid and accurate and has been applied to a variety of formulated products containing chlorhexidine.

THE antibacterial drug chlorhexidine (1:6-di(N'-*p*-chlorophenylbiguanidide N^5)hexane) may be incorporated in various formulations in a range of concentrations. When present in relatively large amounts it may usually be estimated by extraction of the base followed by titration in a non-aqueous medium. Alternatively a spectrophotometric method may be applied to a suitably purified extract. If the concentration of chlorhexidine is 1 per cent or less, however, large amounts are required for the base extraction method and it is often difficult to prepare extracts which are sufficiently free from irrelevant absorption for a spectrophotometric determination to be possible. During a search for a more suitable procedure it was noted that hypochlorous acid reacts with chlorhexidine to give a transient red colour and it was thought that this might form the basis of a colorimetric method of assay.

EXPERIMENTAL

A solution of chlorhexidine diacetate in dilute hydrochloric acid was prepared and diluted with water to contain 0·3 mg. of chlorhexidine per millilitre. Aliquots each of 5 ml. were treated separately with increasing amounts of a solution of sodium hypochlorite containing 1 per cent available chlorine and the effect assessed visually. Small volumes of hypochlorite gave an immediate blood-red colour which faded rapidly to pale yellow; with increasing volumes the blood-red colour persisted but decreased progressively in intensity. This effect was found to be due to the raising of the pH, caused by the alkali present in the sodium hypochlorite solution which precipitated chlorhexidine base before the reaction was complete. The colour produced in neutral or acid solution was too transient to be of practical value and an attempt was made to prevent the precipitation of chlorhexidine base in alkaline solution by the addition of a surface-active agent. With this object 5 ml. of a 20 per cent sodium lauryl sulphate solution was added to a 5 ml. aliquot of the chlorhexidine acetate solution, together with sufficient 0·1N sodium hydroxide solution to produce an excess alkalinity of 0·5 ml. On addition of 5 ml. of sodium hypochlorite solution an immediate blood-red colour was produced which faded very slowly. A slight turbidity was present, however, which

made the solution unsuitable for optical measurement and, since filtration led to adsorption of some of the colour to the filter paper, a search was made for a more suitable surface-active agent. Various compounds of anionic, cationic and non-ionic types were tried, involving about twenty compounds in all. Most of these were effective to a greater or lesser extent but cetrimide and phenactide (*β p-tert.*-octylphenoxyethyldiethylbenzylammonium chloride) were superior to the others, both in clarity of the resultant solution and the stability of the colour. On account of its ready availability cetrimide was chosen and was used in all subsequent work. Sodium hypochlorite solution is inconvenient to prepare and at this stage the more readily prepared hypobromite was tried as a substitute. Initial results were promising and it was decided to investigate the potentialities of the method using both reagents.

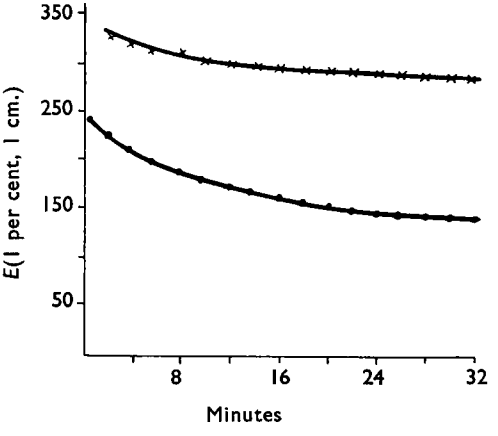

FIG. 1. Stability of colour with time.

X Alkaline sodium hypobromite 1 per cent w/v available bromine.

● Alkaline sodium hypochlorite 1 per cent w/v available chlorine.

Selection of Optimum Conditions of Reaction

Stability of colour on standing at 20°. Aliquots of 5 ml. of chlorhexidine acetate solution were diluted to about 80 ml. in each of two 100 ml. flasks and 5 ml. of 20 per cent cetrimide solution added. After the addition of 0·5 ml. 0·1N sodium hydroxide the colour was developed by adding 5·0 ml. of sodium hypochlorite solution to one, and 5·0 ml. of sodium hypobromite solution to the other, both solutions having 1 per cent of available halogen. The volume of each was adjusted to 100 ml. and the optical density measured at 480 mμ in 1 cm. cells at intervals over the succeeding 30 minutes. Results are shown in Figure 1 and illustrate the superiority of sodium hypobromite solution. In view of the enhanced stability of the colour formed with the latter reagent further work with sodium hypochlorite was abandoned. A standing time of 25 minutes was chosen as sufficient for the colour to reach stability.

Variation of sodium hypobromite concentration. The development of the colour was carried out as described in the preceding section varying the quantity of sodium hypobromite solution, the optical density of each solution being measured 25 minutes after the addition of the reagent. Results are given in Table I and show that variation of the sodium hypobromite content has no effect on the resultant colour.

Variation of alkalinity. The alkalinity of the sodium hypobromite solution was varied between 0·7 and 1·5N, other conditions remaining unchanged, without affecting the optical density of the colour produced. It was convenient to prepare the reagent in 1N sodium hydroxide and this concentration of alkali was used in all subsequent experiments.

Variation of the cetrimide concentration. The reaction was carried out using varying concentrations of cetrimide and the results are shown in

TABLE I

EFFECT OF VARIATION OF THE SODIUM HYPOBROMITE CONCENTRATION ON THE INTENSITY OF THE COLOUR

ml. of sodium hypobromite 1 per cent available bromine	1	2	3	4	5	6
E (1 per cent, 1 cm.) 480 mμ ..	302	303	303	303	303	304

Table II. It will be seen that for constant results to be obtained a minimum of 5 ml. of a 20 per cent solution must be used.

Variation of temperature. The temperature at which the solutions were allowed to stand during the 25 minutes development time was varied between 5° and 25°, and the results are shown in Table III. It is seen that temperature control is most important if reproducible results are to be obtained. As 20° is a readily attainable laboratory temperature subsequent determinations were performed at this temperature.

TABLE II

EFFECT OF VARIATION OF THE CETRIMIDE CONCENTRATION ON THE INTENSITY OF THE COLOUR

ml. 20 per cent cetrimide solution	1	2	3	4	5	6	7	8
E (1 per cent, 1 cm.) 480 mμ ..	294	294	298	303	308	310	310	311

GENERAL METHOD

On the basis of the foregoing experimental work the following procedure was established for the determination of chlorhexidine in simple solution.

Reagents

Alkaline sodium hypobromite. Dissolve 10 g. of sodium hydroxide A.R. in 400 ml. of distilled water in a 500 ml. flask and add, in small portions, 5·5 ml. of bromine A.R. stirring between each addition until the bromine has dissolved. Adjust the volume of the solution to 500 ml. with distilled water. Standardise the solution as follows. Pipette 10·0 ml. into a 250 ml. conical flask and add, in order, 25 ml. of distilled water, 2 g. of potassium iodide (free from iodate) and 10 ml. of glacial acetic acid. Titrate the liberated iodine with 0·1N sodium thiosulphate solution using starch as an indicator.

Based on the determined strength dilute the solution with distilled water to contain 1·5 per cent of available bromine. Prepare the reagent by mixing 66 ml. of the latter solution with 33 ml. of 3N sodium hydroxide solution. It is stable for about one month.

Cetrimide solution. Dissolve 20 g. of cetrimide B.P. in 80 ml. of warm distilled water, cool, and dilute to 100 ml.

Method

Transfer an aliquot of the solution, such as would be expected to contain about 1·5 mg. of chlorhexidine, to a 100 ml. volumetric flask and adjust the volume to about 80 ml. by the addition of distilled water. Add 5·0 ml. of cetrimide solution and, if the solution is acid, sufficient N sodium hydroxide solution to render alkaline to litmus paper, plus 0·5 ml. in excess. Mix well, add 1·0 ml. of *iso*propanol to suppress the froth and place the flask in a water bath at 20° ± 2°. When the temperature has reached equilibrium add 2·0 ml. of alkaline sodium hypobromite solution, adjust the volume to 100 ml., mix and replace in the water bath. At the same time perform a blank on the reagents used exactly as described above omitting only the sample. Allow the flasks to remain in the water bath for exactly 25 minutes and then immediately measure the optical density of both sample and reagent blank against distilled water in 1 cm. cells at 480 mμ on a suitable spectrophotometer. Correct the optical density of the sample by subtracting the reagent blank reading. Obtain the chlorhexidine content of the sample by reference to a calibration graph prepared by repeating the above procedure on solutions containing known amounts of chlorhexidine acetate. A suitable graph is obtained using 2, 4, 6, 8 and 10 ml. aliquots of a 0·0003 g./ml. solution of chlorhexidine acetate.

TABLE III

EFFECT OF TEMPERATURE VARIATION ON THE INTENSITY OF THE COLOUR

Temperature °C	5	10	15	20	25
E (1 per cent, 1 cm.) 480 mμ ..	348	338	317	307	294

Application of the Method to Formulated Products

Compound solutions of chlorhexidine. In all samples examined it was possible to use the foregoing method without modification, the effect of added colouring matter being negligible at the dilution used and the usual perfuming agents being without effect on the development of the colour.

Creams and ointments. It is necessary to extract chlorhexidine from the fatty excipients of the sample before the development of the colour with sodium hypobromite. The following technique was found generally applicable. Transfer an accurately weighed quantity of the sample, expected to contain about 30 mg. of chlorhexidine, to a 150 ml. separating funnel with the aid of 20 ml. of distilled water and add 10 ml. of 1N hydrochloric acid. Extract successively with three portions, each of 25 ml. of chloroform, combining the chloroform extracts in a second separating funnel. Wash the combined chloroform extracts with two 10 ml. portions of water, adding these washings to the acid liquid in the first separating funnel. Discard the chloroform extracts. Transfer the combined acid layer and washings to a 100 ml. volumetric flask and adjust

to the mark with distilled water. Determine the chlorhexidine content on a 5 ml. aliquot of the above solution by the method described for simple solutions.

Lozenges. Transfer an accurately weighed quantity of powdered lozenges, expected to contain about 5 mg. of chlorhexidine to a 150 ml. separating funnel with the aid of 20 ml. of distilled water. Add 10·0 ml. of 3N sodium hydroxide and extract with three successive portions, each of 25 ml., of ether, combining the ether extracts in a second separating funnel. Extract the combined ether extracts with three

TABLE IV

SUMMARY OF RESULTS ON SAMPLES EXAMINED

Sample	No.	Chlorhexidine content		
		Nominal	Proposed method	Alternative method
		per cent w/v	per cent w/v	* per cent w/v
Liquid antiseptic	1	0·30	0·30 0·29	0·29 0·29
	2	0·29	0·29 0·28	0·27 0·27
	3	0·30	0·31 0·30	0·30 0·31
	4	0·30	0·29 0·29	
	5	0·30	0·29 0·29	
	6	0·30	0·30 0·30	
	7	0·30	0·31 0·31	
	8	0·30	0·29 0·30	
		per cent w/w	per cent w/w	† per cent w/w
Antiseptic cream	9	1·03	1·05 1·06	1·04 1·02
	10	1·00	0·98 0·96	0·96 0·96
	11	1·00	0·98 1·00	0·98 0·98
	12	1·00	0·97 0·99	0·98 1·00
Obstetric cream	13	1·00	1·04 1·05	1·04 1·04
	14	1·00	0·99 0·97	0·93 0·94
	15	1·00	1·03	
	16	1·00	0·98	
	17	1·00	1·00	
	18	1·00	1·02	
		mg./lozenge	mg./lozenge	
Antiseptic lozenges	19	5·0	4·95 4·95	
	20	5·0	4·95 5·04	
	21	5·0	4·85 5·00	
	22	5·0	4·9 4·9	
	23	5·0	5·0 4·9	
	24	5·0	4·85 4·9	

Samples 1, 2, 9, 10, 13 and 14 were prepared in the laboratory from materials of established purity.

* A spectrophotometric procedure was used, a reagent blank being performed on all materials used in the formulation.

† The procedure used was titration of the extracted base with perchloric acid in glacial acetic acid.

successive 25 ml. portions of 0·1N hydrochloric acid, filtering each in turn through a No. 1 Whatman filter paper into a 100 ml. volumetric flask. Wash the filter paper with 20 ml. of distilled water, adding the washings to the acid layers in the 100 ml. flask. Adjust to volume with distilled water and mix. Determine the chlorhexidine on a 25 ml. aliquot of the latter solution exactly as described for simple solutions of chlorhexidine.

The procedure described above is suitable for lozenges containing the usual excipients and flavouring agents. The presence of a local anaesthetic of the *p*-aminobenzoic ester type, for example benzocaine, does not cause interference, but if a quaternary ammonium bactericide is present the chlorhexidine is not completely extracted and a modified procedure is necessary. Results on this and previously described formulations are given in Table IV.

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