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ACKNOWLEDGMENTS AND ADDRESSES

Received September 5, 1975, from the *Samuel M. Best Research Laboratory, Massachusetts College of Pharmacy, Boston, MA 02115*

Accepted for publication October 23, 1975.

Abstracted from a thesis submitted by Y. H. Lowe to the Massachusetts College of Pharmacy in partial fulfillment of the Doctor of Philosophy degree requirements.

The authors express their appreciation to the Gillette Co. for financial assistance.

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Gravimetric Determination of Chlorhexidine Using Tetraphenylborate Ion

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Abstract □ A precise and accurate gravimetric procedure was developed for the determination of chlorhexidine diacetate, digluconate, or dihydrochloride. Sodium tetraphenylborate solution was the precipitant in an acidic medium (pH 1). Tablets containing both chlorhexidine diacetate and benzocaine also were assayed.

Keyphrases □ Chlorhexidine—gravimetric analysis, tetraphenylborate as precipitant, pharmaceutical formulations □ Gravimetry—analysis, chlorhexidine in pharmaceutical formulations, tetraphenylborate as precipitant □ Tetraphenylborate—use as precipitant in gravimetric analysis of chlorhexidine □ Bactericidals, topical—chlorhexidine, gravimetric analysis, pharmaceutical formulations

Chlorhexidine is a potent topical bactericidal, effective in high dilutions (1). Presently, it is the most efficient drug for the inhibition of dental bacterial plaque (2–4). Chlorhexidine is employed as the digluconate (5), dihydrochloride (6), and diacetate (7), alone and in combinations with neomycin, cetrimide, or benzocaine in liquid and solid pharmaceutical dosage forms.

A literature review showed that few analytical methods (colorimetry and high-pressure liquid chromatography) were used to determine chlorhexidine quantitatively (8–10). The British Pharmacopoeia offers a nonaqueous method, with glacial acetic acid as the solvent system for the analysis of chlorhexidine digluconate aqueous solution (20% w/v) and chlorhexidine dihydrochloride (5, 6). The procedure for the digluconate involves preliminary evaporation to low bulk of a weighed aliquot of the solution; no monograph is provided for pharmaceutical dosage forms.

This report describes the gravimetric analysis of chlorhexidine salts (gluconate, hydrochloride, and acetate) in aqueous acid solution. Sodium tetraphenylborate, a compound that has found extensive application as a reagent for potassium as well as for the identification and determination of organic bases (11), is used as a precipitant. The method also was applied to tablets containing chlorhexidine diacetate and benzocaine in combination.

EXPERIMENTAL

Reagents—The chlorhexidine salt samples were the highest grades of commercially available materials and were used without further purification. Tablets were prepared in house, and their composition was similar to a product[1] marketed in Italy.

For the sodium tetraphenylborate[2] solution (0.6% w/v), an appropriate amount (purity of 99.5%) was dissolved in water and stabilized according to Cooper (12) at pH 8–9.

Procedures—*Chlorhexidine Diacetate or Digluconate*—Weigh accurately about 30–60 mg of chlorhexidine diacetate, or pipet 2 ml of 2% (w/v) chlorhexidine digluconate solution. Transfer into a 150-ml beaker and dissolve with 20 ml of 0.2 *N* HCl. Slowly add, with stirring, 20 ml of 0.6% sodium tetraphenylborate solution and then allow the mixture to stand for 10–15 min.

Filter the precipitate under suction through a previously dried and tared sintered-glass crucible (porosity 4); then wash the residue with three 5-ml portions of water. Dry the crucible and contents to constant weight (4 hr) at 40–45° at a pressure not exceeding 0.2 mm Hg. Each milligram of the dried chlorhexidine tetraphenylborate is equivalent to 0.5459 mg of chlorhexidine diacetate or 0.7834 mg of chlorhexidine digluconate.

Anal.—Calc. for $C_{70}H_{72}B_2Cl_2N_{10}$: C, 73.37; H, 6.33; N, 12.22. Found: C, 73.69; H, 6.46; N, 11.95.

Chlorhexidine Dihydrochloride—Weigh accurately about 20–60 mg of chlorhexidine dihydrochloride and transfer it into a 150-ml beaker. Dissolve with 20 ml of water by warming gently at 80–85° and cool; then add 0.3 ml of 37% (w/w) HCl. Complete the assay as described for chlorhexidine diacetate or digluconate, beginning with: "Slowly add, ..." Each milligram of the dried chlorhexidine tetraphenylborate is equivalent to 0.5047 mg of chlorhexidine dihydrochloride.

Tablets—The declared amounts, in milligrams per tablet, were: chlorhexidine diacetate, 5; benzocaine, 2; magnesium stearate, 15; mannitol, 300; lactose, 100; and sucrose, 578.

Weigh and powder 25 tablets. Weigh accurately, into a previously tared sintered-glass crucible (porosity 4), a quantity of the powder calculated to contain approximately 100 mg of chlorhexidine diacetate. Then add, under suction, three 20-ml portions of ether and discard the filtrate. Dry the crucible and contents at 50° for 10 min and then dissolve, under suction, chlorhexidine diacetate with 70 ml of 0.2 *N* HCl.

Transfer the combined filtrate and washings of the büchner flask (20 ml of water) into a 100-ml volumetric flask and dilute to volume with water. Pipet an accurately measured aliquot (50 ml) of the solution into a 150-ml beaker. Complete the assay as described for chlorhexidine diacetate or digluconate, beginning with: "Slowly add, ..." Each milligram of the dried chlorhexidine tetraphenylborate is equivalent to 0.5459 mg of chlorhexidine diacetate.

Estimation of Maximum Value for K_{sp}—The apparent solubility product value of chlorhexidine tetraphenylborate was determined in an aqueous hydrochloric acid solution (pH 1) by measurement of the molar detection limit for chlorhexidinium ion in the presence of

[1] Visan, Angiolini S.p.A., Milan, Italy.
[2] E. Merck, Darmstadt, Germany.

Table I—Gravimetric Assays of Chlorhexidine Salts with Tetraphenylborate Solution

Drug	Amount Taken, mg	Recovery, %	*SD* on 15 Analyses, %
Chlorhexidine digluconate	40.0	99.69	±0.42
Chlorhexidine diacetate	28.6–59.6	100.11	±0.46
Chlorhexidine dihydrochloride	22.3–59.3	100.34	±0.31
Tablet (chlorhexidine diacetate)	5.0	97.66	±0.69[a]

[a] Based on six analyses.

a known excess of tetraphenylborate ion. The method is similar to one described by Loach (13).

The apparent solubility product value was calculated from the product of the nominal tetraphenylborate-ion concentration (1×10^{-3} *M*) and the nominal chlorhexidinium-ion concentration (1×10^{-3}–1×10^{-7} *M*) at the detection limit. By using this technique, the solubility product was estimated to be less than 6.5×10^{-13}. While the result could only be regarded as a crude approximation, it provided guidelines for the design of experimental conditions for gravimetric procedures.

RESULTS AND DISCUSSION

As shown in Table I, gravimetric procedures for chlorhexidine salts gave precise and accurate results. The experimental conditions were chosen on the basis of preliminary investigations. The precipitate is only sparingly soluble in water ($K_{sp} = 6.5 \times 10^{-13}$), and it has a definite and reproducible composition corresponding to the formula $C_{70}H_{72}B_2Cl_2N_{10}$. The compound must be dried at a temperature lower than 50° to avoid decomposition. Gravimetric factors of 0.5459 for chlorhexidine diacetate, 0.7834 for chlorhexidine digluconate, and 0.5047 for chlorhexidine dihydrochloride are favorable.

The effect of acidity on the stability of tetraphenylborate and on the filtration properties of tetraphenylborate salts was tested by other workers (14–17), and published information often contained contradictory statements on the optimum conditions for the precipitation. In general, the precipitate formed under alkaline conditions is poorly filterable; however, excessive acidity promotes decomposition of the precipitant (18).

For the determination of the chlorhexidine salts, the final acidity of the reaction solution must be about 0.1 *N* (at 15–25°) to obtain precipitates that are easily separable by filtration. Under these conditions, 1 mole of chlorhexidine salt reacts with 2 moles of sodium tetraphenylborate. Between pH 2 and 5.5, colloidal precipitates which were difficult to filter were obtained; filter aids (19) were not helpful. Because of the tendency for the tetraphenylborate to decompose slowly at this proposed acid concentration (pH 1) (14), standing times up to 60 min did not vitiate the determinations, but the precipitate can be filtered off after standing for 10 min.

When assaying tablets containing chlorhexidine diacetate and benzocaine, benzocaine is dissolved out from the powder mass with ether. 4-Chloroaniline, a possible degradation product of chlorhexidine (10), also is eliminated by ether. Specificity experiments for other interfering products were not conducted. Excipients such as agar and povidone interfere with the proposed method by acting as peptizing agents.

The present method appears to offer a convenient alternative to the few existing methods for the determinations of chlorhexidine salts.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 3, 1975, from the *Istituto di Chimica Farmaceutica dell'Università di Firenze, Via G. Capponi 9, 50121 Firenze, Italy.*

Accepted for publication August 26, 1975.

The authors thank Dr. G. Pisaturo for his assistance.

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