

# FIA determination of chlorhexidine by means of the precipitation with Cu(II)\*

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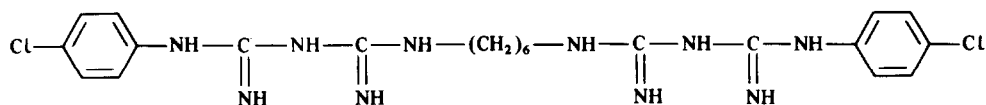
**Abstract:** The determination of chlorhexidine in pharmaceutical formulations is carried out using flow injection analysis (FIA) with measurement by atomic absorption spectrometry (AAS). The method is based on the formation of a copper–biguanide complex precipitate when the sample is injected into an ammoniacal copper solution. The precipitate is retained on a plastic or paper filtering device. A nitric acid stream dissolves the precipitate and carried the Cu(II) to the AAS detector. The chlorhexidine is determined over the range 5–20 ppm. The influence of interfering substances is investigated.

**Keywords:** FIA; pharmaceutical analysis; chlorhexidine.

## Introduction

Biguanides are found in several pharmaceutical formulations and industrial samples. Chlorhexidine, a bactericidal drug, is a member of the biguanide family. It is also found in lens cleaning solutions, cosmetic formulations, deodorants and other industrial products [1].

Liquid–liquid distribution of ion-association compounds and their photometric detection is a sensitive and precise technique widely used. There are reports in the analytical literature on ion-pair compounds of chlorhexidine and dyes or other counterions [2, 3]. Estimation of chlorhexidine according to pharmacopoeias is suitable for the determination of relatively large amounts of the drug [4] but selectivity is poor because of final measurement based on perchloric acid titration in acetic medium. Other published techniques for chlorhexidine determination involve polarography [5], high-performance liquid chromatography [6], gas chromatography [7] and mass fragmentography [8].



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The use of FIA assemblies has also been proposed, the analysis being based on the turbidimetric detection of the system chlorhexidine–bromocresol green–triton X-100 [9] or chlorhexidine–thymol blue [10]. Other examples of FIA procedures using turbidimetric detection may be found in the analytical literature [11–13], but the separation of precipitates in FIA conditions has been approached with a comprehensive study of the variables involved for three different types of the precipitate [14]. The same laboratory [15] has studied the preconcentration and determination of trace amounts of copper with rubeanic acid in silicate rocks.

The method in the present paper for the determination of chlorhexidine in pharmaceutical formulations is based on the precipitation of a copper–biguanide complex in an ammoniacal medium. The copper is dissolved in nitric acid and measurement is by atomic absorption spectrometry (AAS). The biguanide–copper precipitation has previously been proposed for chlorhexidine determination using a conductimetric titration [17].

## Experimental

### Reagents

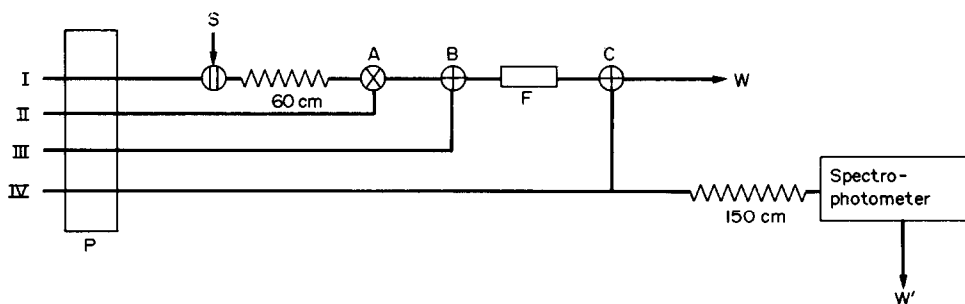
Aqueous solutions were prepared with distilled and deionized water and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  a, r, from Panreac;  $\text{NH}_3$  a, r, from Panreac; nitric acid a, r, from Merck.

### Apparatus

The FIA assembly was provided with a peristaltic pump Minipuls 2 from Gilson; injecting valve, Reodhne 5021; selecting keys from Omnifit; atomic absorption spectrometer SP 1900 from Pye Unicam and Cu cathodic lamp from Pye Unicam. The filtering device made of Teflon from Omnifit or home made using gravimetric paper  $0.8 \text{ cm}^2$  from Selecta, No. 3. The Teflon coil of 0.5 mm i.d. The complete FIA assembly is shown diagrammatically in Fig. 1.

### Procedures

The sample,  $89 \mu\text{l}$  of chlorhexidine in distilled water, was injected into the carrier–reagent stream (0.35 M ammonia and 0.001 M copper solution flowing at 0.7 ml



**Figure 1**

FIA assembly for chlorhexidine determination: I, carrier–reagent stream, 0.01 M Cu(II) and 0.35 M  $\text{NH}_3$ ; II, 0.35 M  $\text{NH}_3$  for washing the precipitate; III, 1.0 M  $\text{HNO}_3$  for dissolving the precipitate; IV, 1.0 M  $\text{HNO}_3$  for the base-line. A, B and C, selector-channel keys; P, peristaltic pump; S, injector; W and W', waste; F, filter.

min<sup>-1</sup>). The precipitate is retained on the filtering device which is washed for 4 min with 0.35 M ammonia solution at 3.5 ml min<sup>-1</sup>. A nitric acid stream (1.0 M), 4.0 ml min<sup>-1</sup>, dissolves the precipitate leading the copper ions to the detector to be monitored at 324.8 nm.

## Results and Discussion

The reaction, Cu(II)-chlorhexidine was investigated in static conditions in order to select the experimental parameters for obtaining a precipitate, suitable for filtration and washing, followed by adequate fast and quantitative acid dissolution. A pink solid is instantly observed after adding Cu(II) to a chlorhexidine solution in a basic medium. The pH of the chlorhexidine solution must not be over 11 because a white solid of free base appears. The pink solid copper complex with chlorhexidine shows 1:1 stoichiometry. A similar compound is observed when Ni(II) solution is added to a biguanide solution. There is some controversy as to the molecular structure of the biguanide-metallic ion complexes [16]; generally they are of cationic nature giving insoluble compounds by paired association with anions (OH<sup>-</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, etc.) present in the solution.

An ammoniacal solution of Cu(II) 10<sup>-1</sup> M was added to an aqueous chlorhexidine solution 1.73 × 10<sup>-4</sup> M giving a final concentration of Cu(II) of 10<sup>-3</sup> M. The ammoniacal concentration was found to be critical for the formation of the chlorhexidine-copper precipitate and 0.35 M was chosen for experience (see later). Lower ammonia concentrations resulted in the coprecipitation of copper basic salts and higher concentrations were not suitable due to the formation of white solid free base. Copper sulphate produced further precipitates than did nitrate or acetate salts, improvement in precipitation was observed with higher copper concentrations but longer washing is required. With the reported conditions chlorhexidine was detectable in the filtrate.

Several different FIA assemblies were tested. Manifolds designed to monitor the excess of copper concentration in the carrier stream after the precipitation were not suitable; the results tended to measure the dilution of copper rather than the biguanide-copper reaction itself. Effort was therefore concentrated on measuring the Cu(II) in the precipitate. The sample cannot be injected into the NH<sub>3</sub> solution because of the tendency to precipitate the white base, it must be injected into a carrier such as the solution of Cu(II) which later merges with the ammonia solution. Alternatively a mixed solution Cu(II)-NH<sub>3</sub> may be used, where the presence of cupric ions avoids the precipitation of the white chlorhexidine base. This last configuration produces a simpler FIA manifold.

The concentrations of both Cu(II) and NH<sub>3</sub> in the carrier solution are critical, they were tested either in static and continuous flow procedures. The Cu(II) concentration was studied up to 0.0138 M, and 0.001 M was selected as the most suitable for further work on the basis of the better output differences between Cu(II) in the precipitate and Cu(II) adsorbed (blank injections). The NH<sub>3</sub> concentration should be selected to give the best compromise between ideal complex precipitation and the undesired precipitation of basic copper salts; the selected value was 0.35 M NH<sub>3</sub>. The dissolution of the precipitate by means of a nitric acid solution was studied over the range 0.1–1.0 M. It appeared to be noncritical, as judged by peak height from 0.5 to 1.0 M, and 1.0 was selected as the value giving minimal standard deviation.

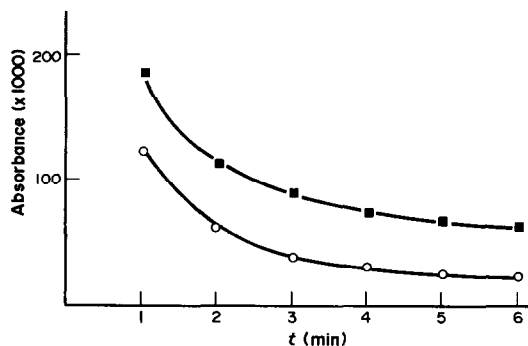
High and poorly reproducible results were obtained if the precipitate was not washed, probably due to the Cu(II) adsorbed on the precipitate and on the filtering device.

Washing is therefore necessary and the washing time is critical especially when a non-Teflon filter is used. Filters tested include sintered glass, Teflon and gravimetric paper (paper supported by a pair of connecting fitting cones). Teflon filters were found to be preferable because of low copper retention and shorter required washing time. The influence of washing time on AAS absorbance is shown in Fig. 2: 4 min is the minimum time required with ammonia solution of 0.35 M and using gravimetric filter paper. With a Teflon filter only 2 min are required.

The FIA parameters were studied by the univariate method and the findings are given in Table 1. The influence of the sample volume on the output was studied up to 250  $\mu\text{l}$  and the findings were linear up to 200  $\mu\text{l}$ . Reproducibility was not affected and the volume was selected which gave the minimum precipitate with washing and a dissolution time as short as possible. The presence of the filtering device gives high resistance to the flow resulting in low flow streams being available. The precipitation is a fast reaction and no differences were observed in the peak heights when the reaction coil length was varied. The flow rate of the acid is important, low flow rates resulted in wider and lower peaks.

#### Analytical application

The proposed FIA assembly gives a linear calibration over a chlorhexidine content range 5.0–20.0 ppm. The equation  $y = 4.0 + 1.98x$  ( $y$  = peak height in mm;  $x$  = chlorhexidine concentration in ppm) with correlation coefficient 0.9992. The reproducibility and sample throughput were found by injecting samples of 10.0 ppm chlorhexidine. The calculated RSD was 3.6% and the sample throughput (deduced from



**Figure 2**

The influence of washing time on AAS absorbance: ○, 0 ppm; ●, 20 ppm of chlorhexidine.

**Table 1**

Optimization of FIA parameters. Studied ranges and selected values

Parameter	Studied range	Selected optimum value
Sample volume	9–260 $\mu\text{l}$	89
Reaction coil length	30–120 min	60
Washing time	1–6 min	4
Flow rate precipitation	0.68–0.72 $\text{ml min}^{-1}$	0.7
Flow rate, nitric acid	2.5–4.5 $\text{ml min}^{-1}$	4.0
Flow rate washing	2–4 $\text{ml min}^{-1}$	3.5

the required time for each injection with 4 min for washing the precipitate) was 10 injections  $\text{h}^{-1}$ .

The tolerance of the method towards possible interfering substances found in pharmaceutical formulations of chlorhexidine was investigated using solutions containing 20 ppm chlorhexidine with various concentrations of other compounds. Results (interference, ppm; relative error, %) were as follows: sucrose, 600, 2.2; acetyl salicylic acid, 200, 2.5; benzocaine, 200, 1.2; ascorbic acid, 200, 1.8. The method was used to measure the chlorhexidine content of Hibitane tablets (from ICI, Farma). The tablets were powdered and extracted with distilled water. The results obtained from tablets agreed with the content stated by the manufacturer, 4.81 and 5  $\text{mg tablet}^{-1}$ , respectively.

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