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Fluorimetric determination of chloroxine using manual and flow-injection methods

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Abstract

A reliable and highly sensitive method is described for the determination of chloroxine in pharmaceutical preparations. It involves the formation of a complex between chloroxine and aluminum(III) in a micellar medium. The complex is a very fluorescent species, and there is a linear relationship between chloroxine concentration and fluorescence intensity over the range 2.0×10^{-8}–5.1×10^{-5} mol l^{-1}. The limit of detection is 5×10^{-9} mol l^{-1}. The method can be easily adapted to a flow system using a three-channel manifold, the peak height being proportional to the chloroxine concentration over the range 5.6×10^{-7}–5.6×10^{-5} mol l^{-1}. Manual and flow-injection procedures permit the determination of chloroxine in the presence of chlorquinaldol, and have been successfully applied to the determination of chloroxine in pharmaceutical preparations.

Keywords: Alumium(III) complex; Chloroxine determination; Flow injection; Pharmaceuticals; Spectrofluorimetry

1. Introduction

Certain halogen derivatives of 8-hydroxyquinoline have a record of therapeutic efficacy in the treatment of cutaneous fungus infections and also of amebic dysentery. The use of the anti-inflammatory drug hydrocortisone together with halogenated hydroxyquinolines is a well recognised treatment for skin disorders.

Various methods have been proposed for the determination of the halogenated 8-hydroxyquinolines, such as titrimetry in non-aqueous or hydroorganic media [1,2], spectrophotometry [3], fluorimetry [4,5], polarography [6], high performance liquid chromatography [7] and extractive alkylation followed by gas–liquid chromatography [8,9].

Fluorimetric methods of analysis offer great sensitivity and selectivity for the determination of a large number of analytes. Unfortunately, problems of solubility and quenching reactions have limited the application of luminescence in analysis. It is well known that micellar media, primarily those formed by synthetic detergent molecules, can be used in fluorescence analysis to solubilize hydrophobic molecules in aqueous solution and to enhance or otherwise modify the luminescence properties of molecules [10].

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The ability of 8-hydroxyquinoline derivatives to form fluorescent metal complexes and the use of these ligands as fluorogenic reagents for the determination of different metal ions is well known [11]. Most 8-hydroxyquinoline derivative chelates are insoluble in water and current methods involve an extraction into a non-polar organic solvent. Another approach is to use micellar media to solubilize and enhance the fluorescence of these chelates for the determination of both metal ions and the 8-hydroxyquinoline derivatives [5,12–17].

The purpose of this work was to develop a sensitive method for determining chloroxine (5,7-dichoro-8-hydroxyquinoline), which is used topically in infected skin conditions and is applied as a cream in the treatment of dandruff and seborrhoeic dermatitis of the scalp. The determination is based on the formation of a complex between chloroxine and aluminum(III) in a micellar system. The procedure is very sensitive, simple and inexpensive.

The experience gained in these laboratories, where analytical methods are used for processing large numbers of samples, shows that flow-injection analysis [18,19] is a very useful and versatile automated technique for the determination of many inorganic and organic analytes. However, no flow injection (FI) method has been found dealing with the determination of the halogen derivatives of 8-hydroxyquinoline.

The characteristics of the reaction between chloroxine and aluminum(III) in micellar media make it suitable for use in unsegmented flow configurations. The proposed FI method allows a high sampling rate with good sensitivity and reproducibility.

Both manual and FI methods were applied to the analysis of the drug in pharmaceutical preparations.

2. Experimental

2.1. Reagents

All chemicals were of analytical-reagent grade and were used without further purification. Doubly-distilled water was used throughout.

Chloroxine stock solution (0.001 mol l^{-1}) was prepared by dissolving the pure product from Sigma (St. Louis, MO) in a 50% (v/v) acetic acid–water mixture. Working solutions of lower concentration were prepared daily by appropriate dilution of the stock solution with water.

Aluminum(III) stock solution (0.02 mol l^{-1}) was prepared from aluminum chloride (Merck, Darmstadt) by dissolving the product in water.

Acetate buffers of different pH values were obtained by mixing appropriate volumes of 2 mol l^{-1} acetic acid and 2 mol l^{-1} sodium acetate to give the desired pH value between 3.7 and 5.7.

Stock solutions of 0.1 mol l^{-1} sodium lauryl sulphate (SLS), 0.1 mol l^{-1} cetyltrimethylammonium bromide (CTAB), 0.1 mol l^{-1} cetylpyridinium bromide (CPC), 0.001 mol l^{-1} Brij-35, 0.2% (w/v) Triton X-100 (TX100), and 0.2% (w/v) poly(vinyl alcohol) (PVA) were prepared by dissolving the required amounts in water.

2.2. Apparatus

An SLM-Aminco Bowman (Urban, IL) Series 2 spectrofluorimeter was used for recording spectra and making fluorescence measurements; excitation and emission spectra were corrected. A Gilson (Villiers-le-Bel, France) Minipuls-4 peristaltic pump and an Omnifit (Cambridge, UK) rotary value were also used.

2.3. Manifold

The schematic diagram of the instrumental set-up is shown in Fig. 1 with conditions as stated. Except for the pump tubing (Tygon), poly(tetrafluoroethylene) (PTFE) tubing (0.5 mm i.d.) was used throughout the manifold. The sample is introduced into the SLS stream with the aid of a rotary valve with a 95 μl loop. This stream is merged 20 cm downstream with a stream of acetate buffer (pH 4.2) at a PTFE Y-piece and merged again 20 cm downstream with a stream of aluminum(III) at a second PTFE Y-piece. The three merged zones travel 60 cm before passing into the flow cell (Hellma 176.052 QS, inner volume 25 μl).

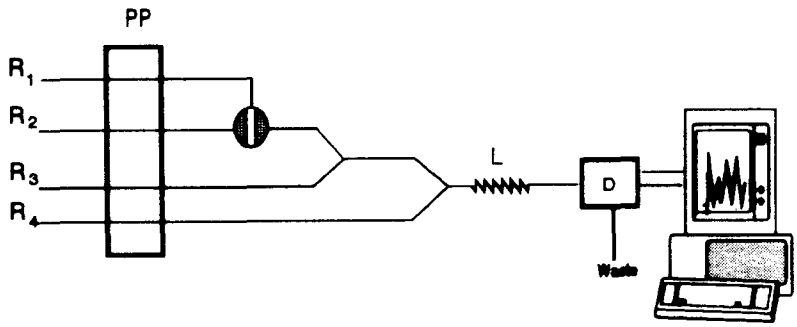

Fig. 1. FI manifold for the determination of chloroxine: PP, peristaltic pump (with flow rate given in ml min^{-1}); R_1, sample; R_2, 8×10^{-3} mol l^{-1} SLS; $R_3 = 0.2$ mol l^{-1} acetate buffer pH 4.2; R_4, 1×10^{-3} mol l^{-1} aluminium chloride; L, reactor coil (length 60 cm, i.d. 0.5 mm).

2.4. Batch procedures

To a 5 ml volumetric flask containing 500 μl of 8×10^{-2} mol l^{-1} SLS, 200 μl of 1.0×10^{-2} mol l^{-1} Al(III) and 500 μl of 2 mol l^{-1} acetate buffer (pH 4.2), the appropriate volume of sample or standard chloroxine solution was added to give a final concentration between 2.0×10^{-8} and 5.1×10^{-5} mol l^{-1}. Before diluting to the mark with distilled water, the pH must be adjusted to 4.2 with 0.1 mol l^{-1} NaOH, if necessary. The fluorescence measurements were made using an excitation wavelength of 399 nm and an emission wavelength of 496 nm.

2.5. FI procedure

The samples, containing between 5.6×10^{-7} and 5.6×10^{-5} mol l^{-1} of chloroxine, were sucked into the sample loop (95 μl) of the injection valve by means of the peristaltic pump and injected into the surfactant stream of the FI manifold. The peaks heights were measured using the flow system and conditions given in Fig. 1. Chloroxine was evaluated from a calibration graph prepared using aliquots of standard chloroxine solutions.

2.6. Determination of chloroxine in pharmaceutical preparations

The tablets or pills (five or more) were finely powdered and weighed. An amount of this powder, equivalent to 20 mg of chloroxine, was accurately weighed and shaken with 25 ml of 4 mol l^{-1} acetic acid. The solution was sonicated in an ultrasonic bath for 10 min, filtered through a Millipore filter, and the filtrate was diluted with distilled water to 1 l in a calibrated flask. An aliquot of this solution was analyzed following the manual and FI procedures described above.

An amount of topical preparation equivalent to 10 mg of chloroxine was weighed and warmed with 25 ml of methanol–acetic acid (60:40) in a water bath with frequent stirring. The mixture was cooled to room temperature, filtered if necessary, and diluted with distilled water to 1 l in a calibrated flask. An aliquot of this solution was analyzed following the recommended procedures.

3. Results and discussion

3.1. Batch method

In preliminary experiments the excitation and emission spectra of several metal–chloroxine complexes in different micellar media and in 25% (v/v) water–ethanol were obtained. The surfactants were tested at a concentration about five times that of the critical micelle concentration (c.m.c.). All measurements were carried out in the presence of an excess of the metal ion because this reflects what could be the situation

in the determination of chloroxine. The results obtained showed that Al(III), Zn(II) and Cd(II) complexes were by far the most fluorescent, and that their fluorescence was always enhanced in micellar media.

The influence of pH on the fluorescence of these three chloroxine–metal complexes was examined over the pH range 3–11 using the optimum excitation and emission wavelengths for each complex. Fig. 2 shows that the greatest fluorescence intensity was given off by the aluminium complex. In addition, aluminum has the advantage that it does not form a fluorescent complex with chlorquinaldol (5,7-dichloro-2-methyl-8-hydroxy-quinoline), the other important chloro derivative of 8-hydroxyquinoline, probably due to the steric hindrance of the methyl group adjacent to the nitrogen donor atom.

The effect of different media on the fluorescent characteristics of the aluminium chloroxine complex is shown in Table 1. The enhancement factors range from 1.5 orders of magnitude for

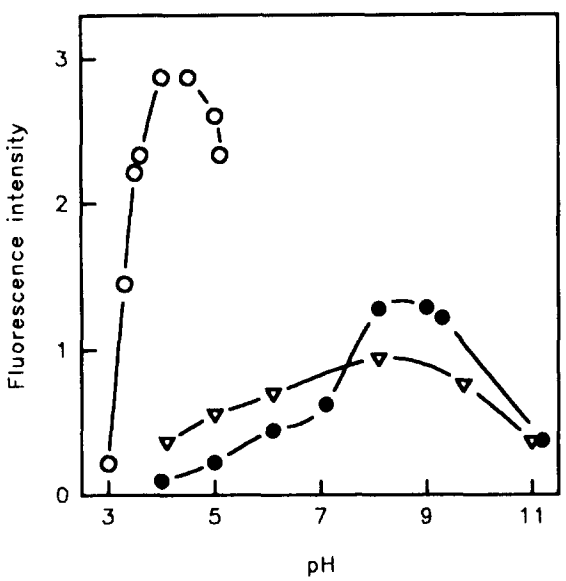

Fig. 2. Influence of pH on the fluorescence intensity of the complexes of chloroxine with Al^{3+} (○), Zn^{2+} (▽) and Cd^{2+} (●) in micellar medium. Conditions: [chloroxine] = 1×10^{-5} mol l^{-1}; [SLS] = 7×10^{-3} mol l^{-1}; [metal ion] = 4×10^{-4} mol l^{-1}.

Table 1
Effect of various surfactants on the fluorescence characteristics of the Al(III)–chloroxine complex

Medium	Relative fluorescence intensity	λ_{ex} (nm)	λ_{em} (nm)
Ethanol–water, 25% (v/v)	100	397	528
TX100, 0.2% (w/v)	5	398	494
Brij-35	12	396	530
PVA, 0.1% (w/v)	202	398	531
SLS, 5×10^{-3} mol l^{-1}	402	399	496
CPB, 1×10^{-2} mol l^{-1}	6	398	494
CTAB, 1×10^{-2} mol l^{-1}	149	396	528

CTAB to about four-fold for SLS micellar media. In all these systems, relatively high surfactant concentrations (about three times the c.m.c.) are required to achieve maximum fluorescence.

On the basis of the results obtained, aluminum(III) and SLS are the most suitable reagents for developing a procedure for the fluorimetric determination of chloroxine taking into account selectivity and sensitivity criteria.

3.1.1. Effect of reaction variables

In order to find the optimum conditions, the influence of pH, the concentration of all reagents and temperature were studied.

The effect of pH on the fluorescence intensity of solutions containing 1×10^{-5} mol l^{-1} chloroxine, 5×10^{-4} mol l^{-1} aluminum(III) and 7×10^{-3} mol l^{-1} SLS is shown in Fig. 2. Maximal and constant fluorescence intensity is attained between apparent pH values of 3.9 and 4.7; pH values greater than 5.5 cannot be used because the complex begins to precipitate. An acetate buffer of pH 4.2 was used in all subsequent experiments. Varying the buffer concentration in the range 0.05–1 mol l^{-1} did not affect the fluorescence intensity.

The influence of aluminum(III) concentration was studied in the range 1×10^{-4}–1.5×10^{-3} mol l^{-1}. As can be seen from Fig. 3A, the fluorescence intensity increased with increasing metal concentration up to 2×10^{-4} mol l^{-1}, but levelled off at higher concentrations. The

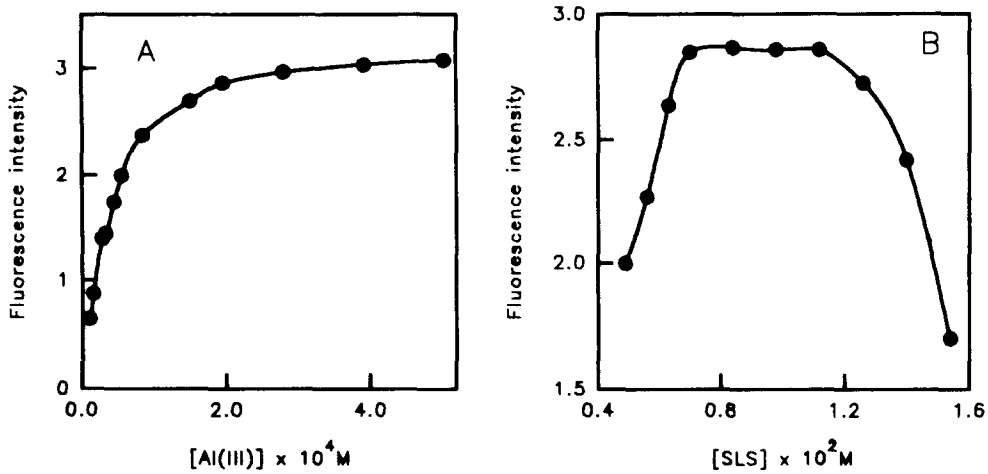

Fig. 3. Influence of aluminum (A) and SLS (B) concentrations on the fluorescence intensity. [Chloroxine] = 1×10^{-5} mol l^{-1} (A); 0.2 mol l^{-1} (B); acetate buffer pH 4.2.

concentration adopted in the procedure (200 μl of 1×10^{-2} mol l^{-1} Al(III) solution in 5 ml) was 4×10^{-4} mol l^{-1}.

The effect of surfactant concentration is shown in Fig. 3B. The fluorescence intensity increased with increasing SLS concentration up to 6.3×10^{-3} mol l^{-1} (about four times the c.m.c.), above which it remained virtually constant up to 1.2×10^{-2} mol l^{-1} but decreased at higher concentrations. The enhancement of fluorescence in the presence of SLS occurs due to increased structural rigidity of the aluminium–chloroxine complex and microviscosity of the medium. The decrease in fluorescence intensity at high surfactant concentration is a fairly general phenomenon [20], which has been explained by the fact that SLS competes with aluminum to bind chloroxine. The SLS concentration selected was 8×10^{-3} mol l^{-1}.

The normal fluctuations of laboratory temperature had a negligible effect on the recovery of chloroxine. All fluorescence measurements were therefore made at room temperature.

3.1.2. Calibration range, sensitivity and precision

Under the conditions recommended in the procedure there is a linear relationship between fluorescence intensity and chloroxine concentration in the ranges 2.0×10^{-8}–4.4×10^{-6} and 4.4×10^{-6}–6.4×10^{-5} mol l^{-1}. The voltage of the photomultiplier tube was adjusted to 800 V for measurements carried out in the lower concentration range and to 550 V for those in the higher concentration range. The detection limit, calculated as the value corresponding to a signal-to-blank ratio of 3, was 5×10^{-9} mol l^{-1}. The precision of the method was established by repeated assays ($n = 11$) using 6.0×10^{-8}, 4.0×10^{-6} and 4.2×10^{-5} mol l^{-1} solutions of chloroxine. The relative standard deviations were 2.3%, 0.62% and 0.39% respectively.

3.2. FI method

In order to increase the precision and sample throughput, FI analysis was used. Several FI configurations were tested with the purpose of providing different reaction conditions in order to obtain the greatest sensitivity. The most suitable arrangement was a three-line manifold as shown in Fig. 1.

3.2.1. Optimization of manifold parameters

The variables studied for optimization of the manifold parameters were volume injected, length of the reactor and flow rate. The reagent concentrations used in these experiments were as follows: surfactant line, 8×10^{-3} mol l^{-1} SLS; buffer line, 0.2 mol l^{-1} acetate buffer pH 4.2; aluminum line, 1×10^{-3} mol l^{-1} $AlCl_3$; and sample solution, 2×10^{-5} mol l^{-1} chloroxine.

Table 2
Determination of chloroxine in pharmaceuticals

Sample[a]	Chloroxine content (mg)		
	Nominal	Batch method[b]	FI method[b]
Synthetic 1	30	29.4 ± 0.3	30.6 ± 0.2
Synthetic 2	20	19.2 ± 0.7	19.5 ± 0.5
Commercial	100	101 ± 1	99.4 ± 0.9

[a] Composition of the samples. Synthetic 1 (ointment): chloroxine, 30 mg; hydrocortisone, 10 mg; paraffin, 1 g. Synthetic 2 (cream): chloroxine, 20 mg; hydrocortisone, 20 mg; oil–water emulsion, 1 g. Commercial: chloroxine, 100 mg; chlorquinaldol, 50 mg; pancreatin, 300 mg; oryza sativa, 60 mg; excipient.
[b] Mean ± standard deviation ($M = 5$).

The volume of sample injected was varied between 35 and 235 μl. The peak heights increased slightly with increasing volumes up to 180 μl, but levelled off at higher volumes. However, a double peak appeared when the loop size was larger than 95 μl: a volume of 95 μl was, therefore, chosen for further experiments.

The influence of the flow rate of each channel on the analytical signal was studied over the range 0.4–2.8 ml min^{-1}. Maximum peak height was obtained at flow rates of 1.2 ml min^{-1} for the SLS line, 0.4 ml min^{-1} for the Al(III) line and 0.4 ml min^{-1} for the buffer line.

Reaction coils measuring 0–100 cm were tested. There was little increase in fluorescence intensity with increased reactor lengths up to 60 cm, above which the signal remained constant. The length chosen for the reactor was 60 cm.

3.2.2. Optimization of reagent concentrations

The effects of varying SLS and aluminum(III) concentrations and the pH of the buffer were tested in the optimized flow system.

The influence of Al(III) concentration was studied over the range 1×10^{-4}–5×10^{-3} mol l^{-1}. The peak height increased steeply with an increase in aluminum concentration up to 2.0×10^{-3} mol l^{-1}, above which it increased only slightly. The concentration selected was 2.5×10^{-3} mol l^{-1}.

The effect of SLS concentration was studied in the range 7×10^{-3}–2×10^{-2} mol l^{-1}. The peak height was maximal and constant between 1.2×10^{-2} and 1.7×10^{-2} mol l^{-1}, and decreased outside this range. The working SLS solution chosen was 1.4×10^{-2} mol l^{-1}.

A 0.2 mol l^{-1} acetate buffer of pH 4.2 was used to adjust the pH to the optimum value.

3.2.3. Calibration graph and figures of merit

With the manifold described, a plot of fluorescence intensity versus chloroxine concentration in the injected sample was linear over the range 5.6×10^{-7}–5.6×10^{-5} mol l^{-1} with a sampling frequency of 60 h^{-1}. The detection limit was about 1.3×10^{-7} mol l^{-1}. The relative standard deviations of 11 injections of each solution containing 4×10^{-6} and 5×10^{-5} mol l^{-1} of chloroxine were 0.38% and 0.29% respectively.

3.2.4. Interferences

The influence of frequently encountered excipients and additives in pharmaceutical dosage forms of chloroxine was studied by preparing solutions containing 1×10^{-6} mol l^{-1} of the drug for the batch procedure and 2×10^{-5} mol l^{-1} for the FI procedure and different amounts of the foreign compound. No interference was found for fructose, galactose, glucose, saccharose, saccharin and cyclamate at (interferent)/(chloroxine) ratios of up to 200/1. Higher concentrations were not assayed. Starch, gelatin and magnesium stearate were tolerated up to 20/1. The tolerance limit was taken as the concentration causing an error of no more than 3% in chloroxine recovery.

Table 3
Recovery of chloroxine added to pharmaceutical preparations

Sample[a]	Added (mg)	Found[b] (mg)	Recovery (%)
Synthetic 1	5.0	4.8 ± 0.07	96.0
	10.0	10.3 ± 0.1	103.0
Synthetic 2	10.0	9.9 ± 0.09	99.0
	20.0	19.7 ± 0.2	98.5
Commercial	50.0	51.2 ± 0.3	102.4
	75.0	74.3 ± 0.2	99.06

[a] For composition, see Table 2.
[b] Mean of three determinations ± SD.

In pharmaceutical formulations, hydrocortisone is usually combined with chloroxine in topical preparations and chlorquinaldol in tablets and sugar-coated pills and so their effects were also examined. It was found that no interference occurred when chlorquinaldol and hydrocortisone were present in a 100- and 30-fold excess respectively.

3.2.5. Determination of chloroxine in pharmaceuticals

To assess the utility of the proposed method several pharmaceutical preparations in different physical forms were analysed. Results are included in Table 2. All values are in good statistical agreement with the nominal values. The recovery was determined by adding various amounts of chloroxine to each pharmaceutical preparation and subtracting the results obtained for pharmaceuticals prepared in a similar manner but to which no chloroxine had been added. The recoveries were in the range 96–103% (Table 3).

4. Conclusions

The results presented here clearly demonstrate that the formation of a complex between chloroxine and aluminum(III) provides a useful fluorimetric method for the determination of this drug. The main advantages of the proposed method are its high sensitivity and simplicity. The absence of interference from chlorquinaldol permits the determination of chloroxine in pharmaceuticals containing both drugs.

A comparison of the batch and FI procedures shows some advantages for the latter, in which the exact control of time allows a better accuracy and reproducibility and the on-line dilution prevents matrix effects. The FI procedure also offers a substantial saving in the time required for the analysis.

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