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Tetracycline, oxytetracycline and chlortetracycline determination by flow injection potentiometry

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Abstract

This paper describes tetracycline (TCH), oxytetracycline (OTCH) and chlortetracycline (CTCH) determination by flow injection potentiometry. In the flow system proposed TC samples are inserted in a carrier solution and converged with a Cu(II) solution of known concentration; the Cu(II) decrease due to its complexation with tetracyclines (TC) was monitored. The detector used was a homogeneous crystalline CuS/Ag_2S double membrane tubular electrode with increased sensitivity. The present system allows tetracyclines determinations within a $48.1–4.8 \times 10^3$ ppm for TCH, $49.1–4.9 \times 10^3$ ppm for OTCH and $51.5–5.1 \times 10^3$ ppm for CTCH and a precision better than 0.4% for the three TC species. This procedure accomplishes 150–200 samples h^{-1} with a Cu(II) consumption of about 13 μg determination^{-1}.

Keywords: Tetracyclines; Cu(II) tubular electrode; Increased sensitivity; FIA

1. Introduction

The fast advances in pharmaceutical industry impose the development of more rigorous analytical methods, particularly faster and inexpensive, for the quality control of pharmaceutical products.

Tetracyclines are amongst the most essential antibiotic families characterized by their wide range of antibacterial effect [1]. This pharmacological family is used in human pathologies as well as in veterinary medicine, animal nutrition and feed additives for cattle breeding.

The biological method of evaluation of microbiological potency is commonly recognised as official method for its determination in pharmaceutical preparations [2,3]. However, the fact that it is time-consuming and of difficult operation as well as subject to random error due to the inherent variability of biological responses, led to the development of other methodologies, particularly based on batch procedures with spectrophotometric detection [4–6].

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The requirements demanded by pharmaceutical industry concerning automation and higher sampling rate led to the promotion of flow injection systems for TC determination. Therefore flow systems with spectrophotometric [7–11], amperometric [12–14], chemiluminescence [15–17] and potentiometric [18] detection were developed. The automated flow injection analysis systems with spectrophotometric detection referred in literature for TC analysis were based on their indirect determinations by monitorization of the coloured complexes formed by tetracyclines with Fe(III) [7,8], WO_4^{2-} [9], 4-aminophenazone and hexacyanoferrate (III) [10] and Cu(II) [11]. These systems present as main disadvantages their application to a very limited concentration range, the need of a compensation procedure for the measurements due to the samples intrinsic colour, and also the fact that, when referred [7,10] they accomplish very low sampling rates, of about 17 [7] and 70 samples h^{-1} [10]. Similar sampling throughputs are also referred for the amperometric FIA systems described [12–14], despite their high analytical sensitivity. Chemiluminescence flow systems [15–17] using hydrogen peroxide/potassium persulfate pair [15], bromine [16] or *N*-bromosuccinimide [17] as oxidising agents are proposed as a good alternative. Nevertheless, these methodologies are very expensive and the oxidising reagents used are highly toxic.

Potentiometric detection FIA systems have been widely used in the analysis of pharmaceutical preparations due to their low cost, easy operation and automation [18]. TC-sensitive conventional electrodes for direct determinations have been previously considered by some authors [19] as an alternative to microbiological procedure although the results showed poor reproducibility.

As organic molecules containing N or O atoms can actuate as electron donors, are able to form complexes with metal ions, which are electron acceptors, if the equilibrium constant is sufficiently high [20], these compounds can be determined by titration with metal salts followed by the appropriate metal ion-selective electrode [18].

The development of tubular electrodes of increased sensitivity [21] presented both the capacity of being steadily adapted to flow systems and improved operational characteristics. In order to determine tetracyclines in pharmaceutical products by using an accurate, fast, simple and inexpensive method, a flow system incorporating a Cu(II) tubular detector with improved characteristics was used to monitor the complexing reaction between TC and copper(II) cation.

2. Experimental section

2.1. Reagents and solutions

All solutions were prepared with bideionized water (specific conductivity less than 0.1 μs cm^{-1}) and with reagents of analytical-reagent grade without further purification.

The stock solution of tetracyclines (Sigma), as the hydrochlorides, was daily obtained by carefully weighing the solid and was kept in a refrigerator before and after use. The TC standard solutions were daily prepared from the previous solution by careful dilution.

Copper nitrate stock solution was obtained by weighing the solid and standardised by potentiometric titration against a standard EDTA solution (Titriplex III, 0.1 M Merck 109992).

2.2. Apparatus

The potential was measured with a CRISON 2002 potentiometer (sensitivity of ± 0.1 mV) connected to a Kipp & Zonen BD 111 recorder.

The solutions were pumped by a Gilson Minipuls 2 peristaltic pump and samples were inserted through a Rheodyne 5020 injection valve. The components of the FIA system were connected with PTFE tubes (0.8 mm i.d.). Auxiliary laboratory-made devices, namely joints, grounding electrode, tubular and reference electrode supports, were used and constructed as previously described [22].

The homogeneous crystalline Cu(II) double-membrane tubular electrode construction and evaluation are described in [21]. An ORION 90-00-02 AgCl/Ag double junction electrode, with a 10% KNO_3 solution in the outer compartment, was used as reference electrode.

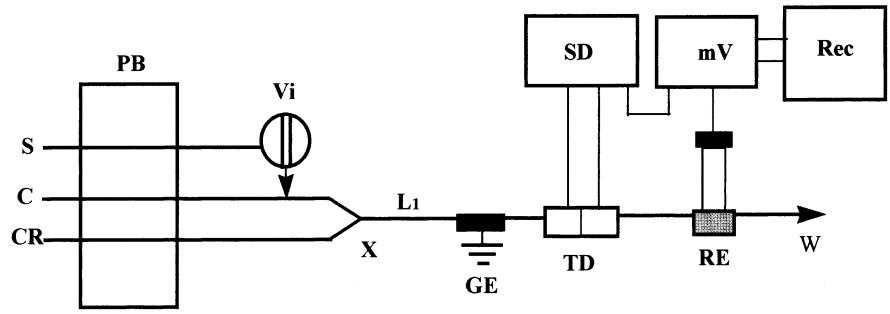

Fig. 1. FIA manifold for the evaluation of TC in pharmaceutical preparations. PB, peristaltic pump; V_i, injection valve; L1, reaction coil (35 cm); S, sample; C, carrier solution; CR, complexing Cu(II) solution; X, confluence; GE, ground electrode; TD, tubular detector; RE, reference electrode; SD, summing device; mV, voltimeter; Rec, recorder; W, waste.

The summing of the potentials of the membranes comprised in the tubular potentiometric detector was carried out by means of a laboratory-made summing device similar to that described elsewhere [21].

A Pye-Unicam SP6-500 UV spectrophotometer was used for the reference method.

2.3. Sample preparations for TC determination

Potentiometric determinations were carried out in different pharmaceutical formulations of TC available in Portugal. For solid samples (coated tablets and capsules) powdering and homogenisation were performed after determination of the average weight. For ophthalmic ointment a quantity equivalent to 50 mg TC was added to 1×10^{-2} M HCl solution which was then kept for a few minutes in a 37°C ultra-sonic bath to allow dispersion and dissolution of the dosage form contents in the aqueous solution. The veterinary powder and ophthalmic solution were analysed after homogenisation of the packet contents. Afterwards, the different samples were diluted in bideionised H_2O to obtain solutions of about 7×10^{-4}–1×10^{-3} M, within linear concentration range of the Cu tubular electrode.

Recoveries of potentiometric measurements were obtained by using the specified methodology, after addition of 0.3–0.5 ml of a 1×10^{-2} M TC solution to 25 or 50 ml sample solutions, in order to achieve a TC concentration of about 7×10^{-3}–1×10^{-3} M.

The quality of results obtained by FIA was also assessed by comparison with those given by the corresponding procedure described in Pharmacopoeia Helvetica [4]. According to Pharmacopoeia Helvetica samples were diluted in a 1×10^{-2} N HCl solution and their absorbance measured by spectrophotometry at 245 nm (TCH), 254 nm (OTCH) and 280 nm (CTCH).

3. Results and discussion

Considering the good working characteristics of CuS/Ag_2S double membrane tubular electrodes, namely increased sensitivity, calibration slope of 62 mV dec^{-1}, lower limit of linear response of 5×10^{-5} M and wide pH operational range (3.0–12.0), a flow system for TC determination in pharmaceutical products was attempted.

Therefore, a FIA manifold with potentiometric detection was established and then optimised (Fig. 1). The sample (200 μl) was inserted in a carrier solution (KNO_3 and HNO_3), converging at X with a $Cu(NO_3)_2$ complexing solution. The variation of Cu(II) concentration, caused by the complexing reaction of TC with Cu(II) solution, was monitored downstream by the potentiometric double membrane tubular detector sensitive to Cu(II).

The manifold was optimised regarding the influence of hydrodynamic and physic-chemical parameters to facilitate TC determinations within a wide concentration range and with the highest

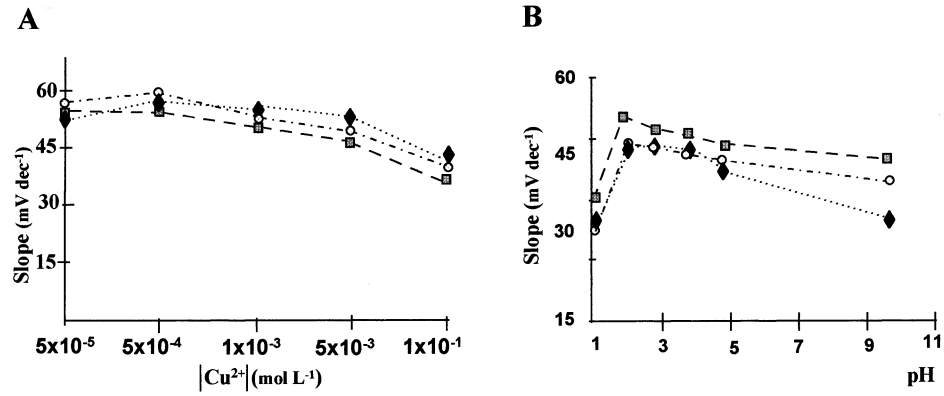

Fig. 2. Variation of the FIA system analytical sensitivity in relation to Cu(II) concentration (A) and pH (B) variation for each of the TC evaluated and (◆, OTCH; ▩, TCH; ○, CTCH).

possible sensitivity (mV dec^{-1} of activity or concentration) and sampling rate. Hence, consecutive calibrations were performed to obtain a linear relationship between peak height and logarithm of copper (II) concentration, for average TC concentrations of the different pharmaceutical preparations available.

The selection of copper cation concentration in the complexing solution affected the potential difference required for the tubular detector to perform the measurements (Fig. 2A), and restrained the TC concentration levels to be determined so, the effect of different $Cu(NO_3)_2$ concentrations (CR), ranging from 5×10^{-5} to 0.1 M, was studied. Copper concentration levels higher than 5×10^{-3} M affected the measurements on samples with TC concentrations inferior to 7.5×10^{-4} M, originating a very low peak height of the analytical signal. For copper concentrations lower than 5×10^{-5} M the potential variation of the most concentrated solutions was insignificant due to insufficient titrant solution to promote the reaction. Besides the baseline potentials were unstable and baseline returning was very slow. The optimisation of this parameter was therefore restrained by the TC concentration levels in the prepared samples and hence, a 5×10^{-5} M Cu(II) concentration was selected for TC determination in the 50 to 500 ppm range and a concentration of 5×10^{-4} M for higher TC concentrations (350 to $\sim 5 \times 10^3$ ppm). In both cases a high sensitivity (mV dec^{-1}) was obtained (Fig. 2A).

The pH influence on the development of the complexation reaction was also evaluated, being studied within an interval of 1.0–9.7 pH units (Fig. 2B). A 0.1 M KNO_3 solution (C) was used as carrier for ionic strength adjustment and different pH values were established by adding HNO_3 or NaOH. The results obtained presented an increase of signal sensitivity as pH diminished what corresponded to a major extent of the complexation reaction in acidic medium that may be related to copper precipitation occurring as oxides and hydroxides, at pH levels higher than 5/6. The low sensitivity obtained at pH 1 might be explained by the fact that Cu(II)-TC complexes' studies demonstrated that complexation majority occur for pH values higher than 2. The highest analytical signal corresponding to a greater extent of the complexation reaction was found at a pH value of 2 for the three TC species studied.

After selection of pH and copper concentrations in the complexing solutions, other parameters such as injection volume, reactor length and flow rate were optimised.

The influence of the injected volume (V_i) was assessed for volumes from 100 to 1000 μl (Fig. 3A). Volumes higher than 500 μl demands higher concentrations for the Cu(II) titrant solution, huge coil lengths (L1) and consequently low sampling rates. With an injection volume of 100 μl low peak height signal was attained, what compromised the determination of analyte concentrations lower than 5×10^{-4} M. Therefore, an

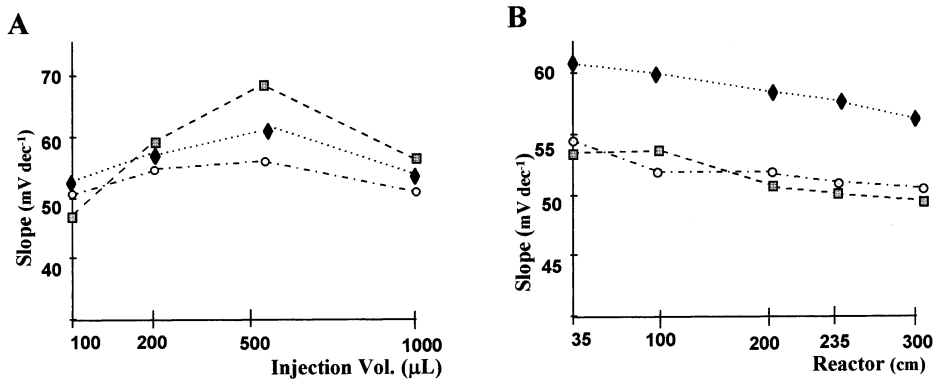

Fig. 3. Variation of the FIA system analytical sensitivity in relation to the injection volume (A) and reactor length (L1) (B) variation for each of the TC evaluated (◆, OTCH; ▩, TCH; ○, CTCH).

injection volume of 200 µl was selected since it allowed the attainment of a good sensitivity and reproducibility without prejudice of the sampling rate and avoiding great sample consumption.

For the fixed injection volume (200 µl) different reactor lengths (35 to 300 cm) from the confluence to the detector (L1) were also studied (Fig. 3B). An optimum value was chosen so that the sampling rate would not be compromised and the total mixing of sample and complexing solution would be assured. A decrease of sensitivity and sampling rate was observed with the increase of the sample dispersion for the three TC species. A reactor of 35 cm, which was the shortest possible length required for the connections between the different components of the FIA system, was enough to promote a complete complexing reaction without compromising the analytical signal sensitivity (peak height), besides providing a good reproducibility with high sampling rates.

The effect of the flow rate was evaluated keeping the same value for both channels (C and CR). The total flow rate was varied from 2.7 to 8.0 ml min^{-1}. No significant analytical signal variation was observed for the interval studied. Therefore, a value of 8.0 ml min^{-1} allowed analytical calibrations with good sensitivity as well as high sampling rates. The FIA system optimised as described above enabled a maximum sampling rate of 200 samples h^{-1} and a minimum of 150 samples h^{-1}, for TC concentrations of 48.1–4.8×10^3 ppm for TCH, 49.1–4.9×10^3 ppm for OTCH and 51.5–5.1×10^3 ppm, for CTCH (Fig. 4).

Reproducibility of the system was assessed throughout a working day by performing six calibrations for about 8 h work. The potential varia-

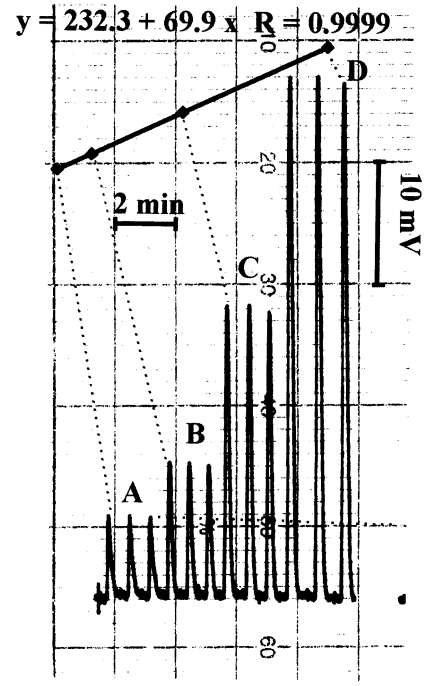

Fig. 4. Recorder output corresponding to the tracing of a calibration curve for OTCH determination and respective calibration equation. OTCH standard solutions injected: A, 6×10^{-4}; B, 7×10^{-4}; C, 1.05×10^{-3} and D, 2×10^{-3} M.

Table 1
Results obtained from TCH, CTCH and OTCH determination in commercial pharmaceutical preparations by the proposed method and reference procedure

Pharmaceutical Preparation	FIA[a]	Reference Proc[a]	Recovery (%)[b]
Terramicina (Veterinary powder)	318 ± 0.01	328 ± 0.01	98.9
Terricil (ophthalmic ointment)	5.30 ± 0.01	5.80 ± 0.01	99.0
Ciclobiótico (capsule)	849 ± 0.01	873 ± 0.01	101
Ciclobiótico (capsule)	761 ± 0.01	734 ± 0.01	104
Neociclina (capsule)	886 ± 0.01	892 ± 0.01	103
Hostaciclina (coated tablet)	550 ± 0.01	563 ± 0.01	104
Aureomicina (ophthalmic solution)	19.8 ± 0.01	20.6 ± 0.02	99.7
Aurecil (ophthalmic ointment)	43.0 ± 0.01	44.6 ± 0.02	99.3

[a] Mean and standard deviation of four determinations for the same sample. Results expressed as mg g^{-1}.
[b] Recovery values of TC obtained by using the specified methodology, after addition of 0.3–0.5 ml of a 1×10^{-2} M TC solution to 25 or 50 ml sample solutions.

tion for TCH, OTCH and CTCH was less than ± 2.6, 1.1 and 1.4 mV day^{-1}, respectively.

3.1. Analytical applications

The usefulness of the proposed method for the assay of commercial TC formulations was assessed by studying the effect of some common excipients used in pharmaceutical preparations. The influence of some inorganic and organic compounds, namely glucose, sucrose, lactose, starch, urea, polyethylene glycol, PEG 4000, Na_2SO_3 and NaCl on the FIA system were evaluated. No interference was observed from any of the excipients tested other than NaCl.

The present FIA manifold was therefore applied to the determination of TC in different pharmaceutical formulations available in Portugal. The results obtained are presented in Table 1. The quality of the results obtained with the FIA system (C_F) was assessed by comparison with the results provided by the reference procedure (C_R) described in Pharmacopoeia Helvetica. A linear relationship $C_F = -1.64(\pm 21) + 0.995(\pm 4.0 \times 10^{-2})\ C_R eR = 0.999$ could then be established, showing a good agreement between methods. Recovery assays performed for all the pharmaceutical samples, presented values close to 100%. (Table 1).

Student *t*-test was also carried out and a theoretical value of 0.696 was obtained for the determination of TC by the proposed method, being less than the fixed value (2.365) for a reliable interval of 95% [23].

The within-run precision of FIA methodology was assessed by calculating the relative standard deviation after performing 12 replicate injections of each of the three samples (one of each type of TC) of 318.0 mg g^{-1} (OTCH), 761.3 mg g^{-1} (TCH) and 19.8 mg g^{-1} (CTCH) concentrations. The values obtained showed a good precision with a relative standard error less than 0.4%.

4. Conclusions

The determination of tetracyclines by using a flow injection system with potentiometric detection proved to be an advantageous method regarding spectrophotometric detection [4–11], since determinations within a wide concentration range, regardless of the samples colour and turbidity, could be accomplished. The potentiometric detection system of increased sensitivity provides improved precision, high sampling rates and better reproducibility than the previously reported [18].

This system facilitated the determination of these compounds with high sampling rates, from 150 to 200 samples h^{-1}, and a low consumption of reagent, about 13 μg Cu(II) $determination^{-1}$.

The results obtained in this work enable to conclude that this methodology can be applied to the analysis of other antibiotics chemically similar

to TC, only requiring the adjustment of the complexing solution copper concentration and pH.

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