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Copper carbonate as a solid-bed reactor for spectrophotometric determination of doxycycline and oxytetracycline in an unsegmented continuous flow assembly*

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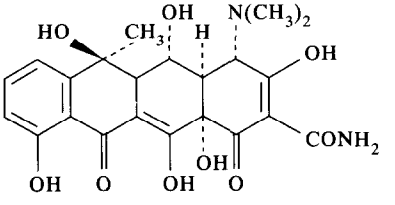
Abstract: The FIA–spectrophotometric determination of doxycycline was carried out by reaction of the drug with cupric ions entrapped in a polymeric material in a packed-bed reactor: the complex formed was then injected into a manifold with an alkaline solution as carrier. The developed colour was monitored at 395.0 nm. The method was applied to the determination of doxycycline in different pharmaceutical formulations. The calibration graph for doxycycline hyclate was linear over the range 10.0–80.0 mg ml^{-1} (n = 8) with a relative standard deviation of 1.4% (at 25 mg ml^{-1}) and a sample throughput of 128 h^{-1}. The proposed procedure was also applied to the determination of oxytetracycline in pharmaceutical formulations.

Keywords: *Solid-bed reactors; FIA; tetracyclines.*

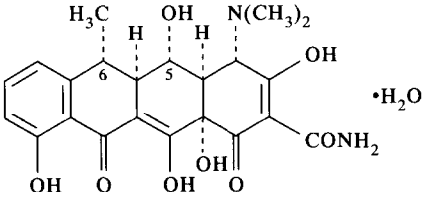
Introduction

Doxycycline is 4-(dimethylamino)-1,4,4a,5,5a,6,-11, 12a-octahydro-3,5,10,12,1a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate; the hyclate is doxycycline hemietanolate hemihydrate [1] (Fig. 1).

Doxycycline is a yellow crystalline powder and is very slightly soluble in water. Its antimicrobial action is similar to that of tetracycline hydrochloride; it is more effective than tetracycline against most species. Doxycycline is readily absorbed from the gastro-intestinal tract and is excreted more slowly than most other tetracyclines; at equivalent doses, it produces higher plasma concentrations than most tetracyclines and these concentrations are maintained for longer periods. Doxycycline is administered in capsules as the hydrochloride or in syrups and suspension as the calcium chelate or monohydrate. The hydrochloride is also given by intravenous injection. Side-effects are common to all tetracyclines; gastro-intestinal effects including nausea, vomiting and diarrhoea are common especially with high doses [2].



Oxytetracycline



Doxycycline

Figure 1
Formulae of oxytetracycline and doxycycline.

Tetracyclines can be determined by microbiological, chromatographic, spectrophotometric, fluorimetric and titrimetric procedures. Official procedures include chromatographic [3] or biological assays of antibiotics [4].

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Reactions proposed for spectrophotometric determination of doxycycline are with molybdate [5], uranyl nitrate [6], cupric chloride [7] and cerium(III) [8].

On the other hand, the use of solid-phase reactors has become one of the most interesting developments in continuous-flow methodologies, including flow-injection analysis. The interest is ascribed to inherent advantages offered over dissolved reagents. One broad category of reagent immobilization is the physical entrapment of the reagent. The present paper is concerned with the strategy of reagent immobilization based on polymerization of linear unsaturated polyester chains [9–11] in order to propose a spectrophotometric procedure for the determination of doxycycline and oxytetracycline. The procedure is based on the reaction of the drug with immobilized cupric ions and the injected solution is then led to the detector by an alkaline solution.

Experimental

Reagents, apparatus and procedures

Reagents. Analytical-reagent grade chemicals were used unless indicated otherwise. Aqueous solutions of doxycline hyclate (Pfizer) were prepared in de-ionized water. Sodium hexametaphosphate (Guinama); sulphamethoxypyridazine (Guinama); α-chymotrypsin (Guinama); sodium hydroxide (Panreac); and hydrochloric acid (Panreac) were used.

The solid bed-reactor was prepared with $CuCO_3$, $Cu(OH)_2$ $2H_2O$ (Panreac). Polyester resin solution AL-100-A (Reposa) contained low molecular-weight polyester chains and a cobalt compound as activating agent of the reaction and methyl ethyl ketone as a catalyst (Akco).

Flow injection assembly. A continuous-flow manifold was used with the column adjacent to the injection valve. A Rheodyne Model 5041 sample injector and a Gilson Minipuls 2 pump were used. The complex formed was determined using a UV–vis spectrometer, model Lambda 16, (Perkin–Elmer) at a wavelength of 395.0 nm and provided with a 18-μl flow cell. PTFE tube coils in the FI assembly were of 0.8 mm i.d.; the i.d. of the PTFE tubing in the bed-reactor was 1.5 mm.

Procedures. The bed-reactor was prepared as described previously [12, 13]. A 16.6 g mass of copper carbonate was added to 17.4 g of the resin solution; after homogenization by manual stirring, 0.4 ml of the catalyst was added and the resulting mixture was stirred before it became solid. The solid obtained was dried at room temperature for 2–3 h, then reduced to small particles which were separated by sieving. The selected (150–200 μm) solid particles were washed with distilled water at 80°C, sieved again and introduced by the aid of a mini-funnel into a 1.5-mm i.d. PTFE tubing.

Sequential optimization of experimental parameters

After pre-optimization of several chemical parameters (pH, acidity and temperature) y modified simplex (MSM) method was used for optimization of flow-injection parameters. The initial simplex method was chosen according to Yarbro and Deming [14] with a side-length of one and the former vertex on the origin of the co-ordinates. The region of the variables was standardized by the modification proposed by Morgan and Deming [15] and a program that required the ranges of any tested variable to be input into the computer was applied in order to obtain the five vertices. The modified simplex program for this work was based on the method of Nelder and Mead [16]. After the first set of experiments within the MSM, a new MSM was applied by adjusting new limits for every tested parameter according to results obtained in the first set. The chemical parameters were finally re-optimized by the univariate method.

Doxycycline determination in pharmaceutical formulations

Capsules. Three capsules were powdered in an agate pestle and mortar and three aliquots of about 0.23 g were each dissolved in 0.1 M hydrochloric acid. The solution was filtered and the volume was diluted to 250 ml with 0.1 M hydrochloric acid. A 10.4 ml volume of 1.0 M NaOH was added to aliquots of 10 ml (pH adjusted to 2.0–2.2) and then were diluted to 100 ml with de-ionized water.

Injection vials. One vial was diluted to 1 l with 0.5 M HCl; aliquots of 10 ml were added to 4.2 ml of 1.0 M NaOH and the solution was diluted to 100 ml with distilled water. The final pH was adjusted to 2.0–2.2.

Results and Discussion

The first few assays were designed to examine the reaction between the immobilized spectrophotometric reagent with two different tetracyclines, doxycycline and oxytetracycline. On the other hand, the versatility of the polymeric solid-phase reactor allowed the immobilization of different reagents; this led to the selection of three different reactions (with uranyl, cerous and cupric ions) of the tetracyclines for suitable continuous flow determination with the aid of solid-phase reactors. Several series of experiments were carried out with the three pre-selected reagents: visual observation; recording absorption spectra in different media; and construction of pre-calibration graphs. Reaction with cupric ions resulted in the most suitable linearity range and sensitivity; 1–24 μg ml^{-1} compared with 10–50 μg ml^{-1} with cerous ions or 20–80 μg ml^{-1} with uranyl. Reaction with cupric salts and an alkaline medium was selected for further work.

Preliminary experiments on the entrapped copper ions with tetracyclines were carried out. Aliquots (5 ml) of doxycycline 3.1×10^{-4} M were added to 20 mg of the immobilized copper carbonate (16.6 g of copper carbonate per 17.4 mg of resin). The UV–vis spectra of the resulting solutions were recorded and compared with the corresponding spectrum of the drug in a basic medium, that is 3.9×10^{-4} M doxycycline at pH 12.2.

Further preliminary tests were carried out with the aid of different flow assemblies in order to select the best manifold-configuration (Fig. 2). The solid-phase reactor was placed in three different points in the sample loop; between the injection valve and the detector; and in front of the injection valve. Transient signals and spectra (300–500 nm) of the resulting solutions were recorded. Solutions circulating through assembly 2B resulted in absorp-

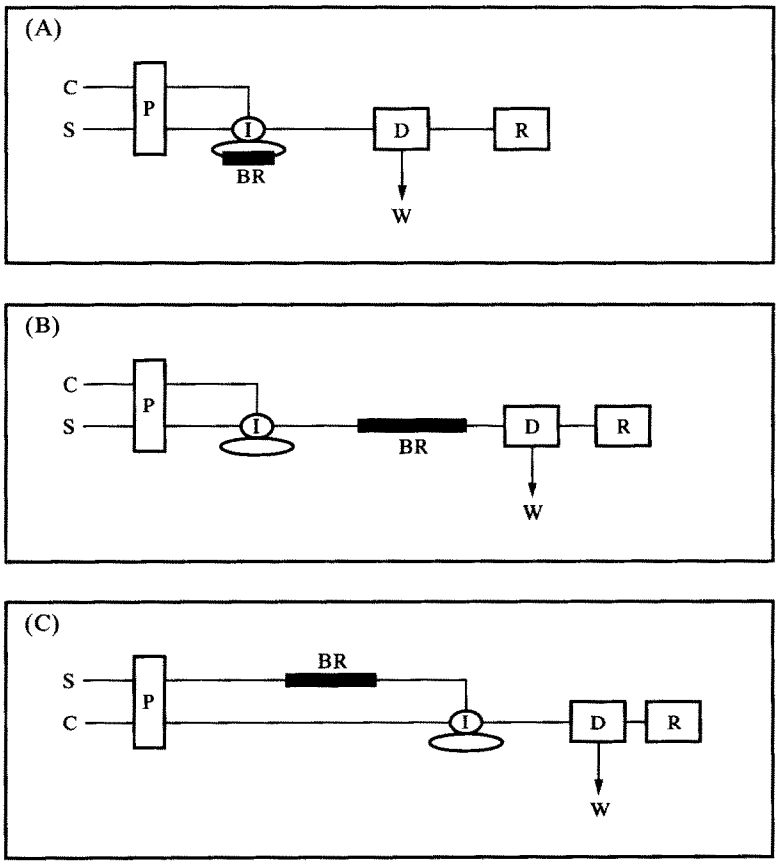

Figure 2
Flow manifolds. (A) and (B) Assemblies tested in preliminary experiments. (C) Proposed FIA assembly for doxycycline and oxytetracycline. S, Sample; C, carrier; BR, bed-reactor; I, injection valve; D, detector; R, recorder and W, waste. (See text for details.)

tion spectra similar to those of solutions of drug in an alkaline medium and without reaction with cupric ions. Assemblies in Fig. 2(A) and (C) provided solutions with suitable spectra (reaction with cupric); degradation of solid reagent in assembly 2A was quickly observed and was probably due to formation of cupric oxide. The flow-manifold in Fig. 2(C) was selected for further work.

Three different concentrations of sodium hydroxide were tested with the aid of the selected assembly: flow rate of sample and carrier streams, 3.72 ml min^{-1}; column length was 8.2 cm; sample volume, 295.5 μl; and injector–detector length, 20 cm. The results (as absorbance at 395 nm) corresponded to NaOH concentration (M) of: 0.2911–0.0277; 0.2969–0.0286; and 0.2938–0.0295. The concentration 0.0286 M was selected for sodium hydroxide.

Simplex optimization of flow-injection parameters

A sequential optimization process was carried out (see Experimental section). Although sub-optimal conditions were obtained when using MSM only for flow-injection parameters, this strategy permitted rapid progress (only quick changes are required for each vertex given by the computer). On the other hand, pre- and post-optimization of the chemical parameters was enough to ensure suitable final conditions. This sequential process permitted a previous knowledge of the kinetic behaviour of the analyte-reagent system under the conditions provided by the continuous-flow assembly. The pre-optimization step (for chemical parameters) allowed development of the MSM optimization procedure by using initial chemical conditions close to the optimum values.

Once a stable chart-recorder baseline had been obtained a sample was injected; the reaction took place and the resulting peak absorbance at 395.0 nm was measured. This was repeated until an RSD <1% was obtained for the peak-height (four or five measurements usually sufficed). The range of variables considered is shown in Table 1. Zero values of peak-height were assigned to the entries which were out of the variable range. After 15 experiments it was decided to test a new simplex by considering a new range of variables, which are also shown in Table 1 and Fig. 3.

After 17 experiments it was decided that the system did not merit further experimentation; in order to obtain the best compromise of

Table 1
Simplex optimization of flow-injection parameters

Parameter	Tested range		Selected value
	First simplex	Second simplex	
Carrier flow rate (ml min^{-1})	0.62–5.38	1.22–5.38	4.85
Sample flow rate (ml min^{-1})	0.62–5.38	1.22–4.19	1.75
Sample volume (μl)	104.2–607.2	154.8–245.2	172.6
Injector–reactor length (cm)	20–100	30–100	45.3

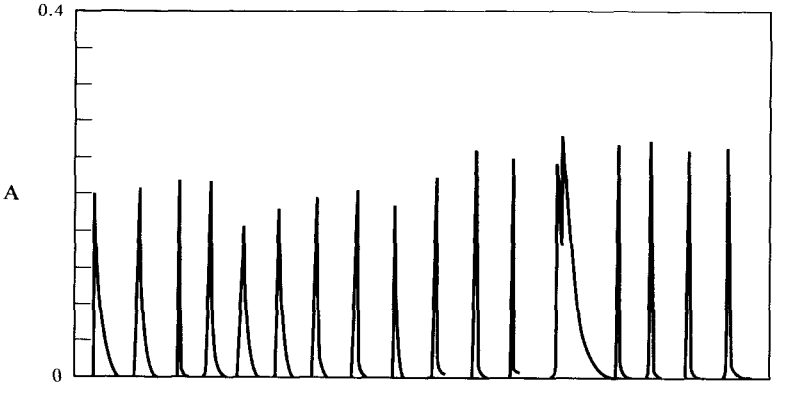

Figure 3
Outputs obtained in the second MSM series.

sensitivity (peak-height), reproducibility (RSD%) and sample throughput, the two points from the simplex producing the highest transient signals were selected and compared by recording series of four injections with different concentrations of doxycycline (10.4, 15.6, 20.7 and 270 mg l^{-1}). The FIA parameters corresponding to the point selected as the best are given in Table 1.

Two parameters (pH and solid-bed reactor length) were re-optimized on the basis of results obtained from the MSM and by means of the univariate method. The column length was tested over the range 2.5–15 cm (15 replicates for each point) and the results showed that it was a relevant parameter; peak height varied from 0.335 to 0.351 (absorbance units). A column length of 8.5 cm was selected for further work.

The pH was re-optimized over the range 1.73–2.47. The pH, absorbance and RSD (n = 15) were, respectively: 1.73, 0.240, 0.5%; 1.92, 0.302, 0.4%; 2.01, 0.384, 0.4%; 2.11, 0.382, 0.6%; 2.21, 0.390, 0.3%; and 2.47, 0.368, 0.7%. The maximum peak-height was observed for the pH range 2.0–2.2, which was selected for further work (Fig. 4).

Analytical applications

A study of the analytical application of the continuous-flow procedure was carried out to establish the application range, reproducibility and sample throughput.

The calibration graph was linear over the range 10–80 mg ml^{-1} doxycycline. The day-to-day stability of the packed-bed reactors was tested by comparing the linear graphs obtained for different time periods up to 6 days. Some of the results obtained are given in Table 2.

A set of 32 different samples containing 2.5 μg ml^{-1} of doxycycline were injected in order to determine the reproducibility (RSD) and sample throughput; the results obtained were 1.4% and 128 h^{-1}, respectively.

The tolerance of the method to foreign compounds which can be found in typical pharmaceutical preparations containing doxycycline was investigated; to solutions containing 25 mg l^{-1} of the drug were added various concentrations of the possible interfering substances up to 100 mg ml^{-1} or when the relative error (by comparison with pure doxycycline solutions, 25 mg l^{-1}) was about 3%. The results in terms of concentration (mg l^{-1}) and relative error were: lysozyme hydrochloride, 100, 3.9%; sodium hexametaphosphate, 100, 0.4%; sulphamethoxypyridazine, 100, 2.4%; and α-chymotrypsin, 100, 0.4%.

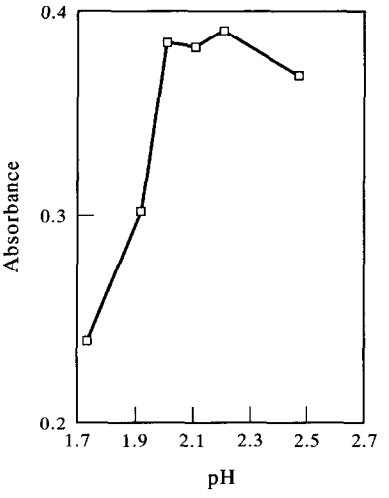

Figure 4
Influence of the pH on the outputs, studied by means of the selected assembly after the optimization of FIA parameters.

Table 2
Calibration equations (day-to-day stability)

Slope	Intercept	Correlation coefficient
Doxycycline* (n = 5)		
0.01204	0.0534	0.9985
0.01217	0.0483	0.9991
0.01188	0.0586	0.9989
0.01207	0.0426	0.9995
Oxytetracycline† (n = 5)		
0.01279	0.0332	0.9990
0.01306	−0.0249	0.9998
0.01426	0.0075	0.9997

*Mean values and RSD: intercept, 0.0507, 0.69%; and slope 0.01204, 0.01%.
†Mean values and RSD: slope, 0.01337, 0.08%; intercept, 0.057, 2.9%.

The doxycycline content of Doxiten BIO capsules (Zyma) were determined. The capsules were treated as described under experimental procedures. At least five different preparations were analysed and the results were compared with those supplied by the manufacturer: declared 100 mg $capsule^{-1}$; found 102.3 mg $capsule^{-1}$, relative error = 2.3%.

The method was also applied to the determination of oxytetracycline. The calibration graph was linear over the range 10–80 mg ml^{-1}; with the equation $A = 0.053 + 0.0136x$ (A = absorbance and x = oxytetracycline concentration in mg l^{-1}) and the corresponding correlation coefficient was 0.9998. The method

was applied to determination of the drug in Terramicina capsules and Terramicina vials (both Pfizer). The results were 244.4 mg capsule^{-1} and 258 mg vial^{-1}; declared 250 mg capsule^{-1} and vial^{-1}. Relative errors were 2.2 and 3.2% for capsules and vials, respectively.

Conclusions

A spectrophotometric procedure has been developed for the determination of some tetracyclines for application to the analytical control of pharmaceutical formulations. The method is based on the reaction of the drug with copper (II) carbonate entrapped in a packed-bed reactor. The use of solid reagents enables a simple FIA manifold and a spectrophotometric detector to be used.

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