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Short Communication

Determination of erythromycin in tablets and capsules using flow injection analysis with chemiluminescence detection

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Introduction

Several methods have been described for the determination of the antibiotic erythromycin (Fig. 1) in pharmaceutical and biological samples. The USP method employs a microbial assay [1]. Although a true measure of antibiotic activity is measured, this method is time-consuming because of the 16–18 h incubation period. Because erythromycin does not have conjugated double bonds in its structure, spectrophotometric quantitation is not straightforward. A UV spectrophotometric method at 236 nm can determine erythromycin after alkaline hydrolysis [2]. Hydrolysis of erythromycin in concentrated sulphuric acid produced a coloured product that could be measured

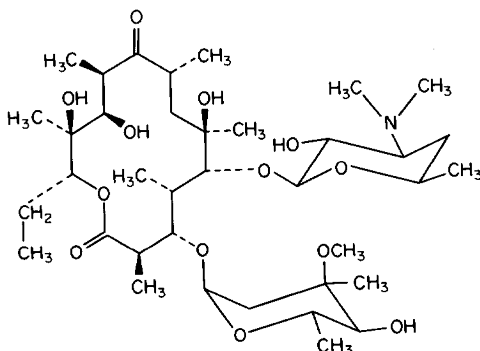

Figure 1
Structure of erythromycin A.

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spectrophotometrically at 482 nm [3]. Another visible spectrophotometric method using 2-nitrobenzaldehyde as a derivatizing agent has been used for dosage forms [4]. Complex formation with bromocresol purple also permitted colorimetric determination of erythromycin [5]. Radioimmunoassay [6] and constant current stripping analysis [7] have also been employed. Although TLC [8] and GC–MS [9] have been used, HPLC is probably the most common chromatographic method for erythromycin. However, with HPLC and UV detection at 215 [10] or 200 nm [11], only mg ml^{-1} concentration levels of erythromycin could be assayed. Fluorescent detection in conjunction with HPLC has been reported [12], but multiple post-column reactions and extraction steps were necessary. Although macrolides such as erythromycin require a high oxidation potential at glassy carbon electrodes (>1.20 V), low detection limits in the ng range were possible using HPLC with dual electrode electrochemical preoxidation and detection at 0.8 V [13, 14]. However, fouling of the electrodes may be a potential problem after extended use.

Using flow injection analysis (FIA), recently it has been reported that aliphatic mono-, di-, and trialkylamines will generate chemiluminescence upon reaction with tris(bipyridine)ruthenium(III) [$(Ru(bpy)_3^{3+}$] [15]. In particular, trialkylamines such as tripropylamine can be detected at levels below 0.1 pmol [16]. Because erythromycin has a tertiary amino group, we have explored this chemiluminescence reaction for the rapid assay of erythromycin in tablets and capsules without prior sample clean-up using FIA.

Experimental

Chemicals

All chemicals used were reagent grade or better. $Ru(bpy)_3Cl_2 \cdot H_2O$ was obtained from Aldrich (Milwaukee, WI) and used without further purification. The triply distilled water was from a Barnstead Nanopure distillation unit (Sybron/Barnstead; Boston, MA). The acetonitrile was HPLC grade (Pierce; Rockford, IL). Erythromycin and corresponding pharmaceutical preparations were purchased from Sigma (St. Louis, MO) and local pharmacies, respectively.

Equipment

The FIA instrument consisted of a Beckman Model 110A pump with a pulse dampener (52 cm × 4.6 cm i.d. stainless steel), a Rheodyne Model 7010 injector (Rheodyne, Berkeley, CA) fitted with a 20-μl sample loop, and a modified Waters 420-AC HPLC fluorometer (Milford, MA). Modification was required because the lifetime of the chemiluminescence reaction was short and therefore mixing of the $Ru(bpy)_3^{3+}$ reagent stream and sample stream should occur in the flow cell. (Specific details of the flow cell design are under patent proceedings by Waters Chromatography Division (Millipore Corp.) [17].) The excitation source of the Waters 420-AC HPLC fluorometer was removed but the position and geometry of the 8-μl flow cell, optics, and the photomultiplier tube remained unchanged. A photomultiplier tube Model R928YP2547 (Hamamatsu; Middlesex, NJ) with maximum sensitivity in the visible light region was installed because the optimum chemiluminescence emission wavelength was 600–625 nm. The oxidized ruthenium complex was delivered to the flow cell using a Elitea C4A peristaltic pump (Seattle, WA).

The $Ru(bpy)_3^{3+}$ solution was filtered through a polypropylene filter (Alltech; Deerfield, IL) to minimize contact with stainless steel which appeared to quench the chemiluminescent signal. A constant temperature circulation unit Model 900 (Fisher

Scientific; Pittsburgh, PA) cooled the chemiluminescent reagent at 0–2°C using a water–ethylene glycol (50:50, v/v) coolant [16].

Procedure

The chemiluminescent reagent stream consisted of a 1 mM $Ru(bpy)_3^{3+}$ solution, made in 10 mM acetate buffer at pH 5.8. The solution was oxidized at +1.35 V from $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ using an IBM Model EC/225 Voltammetric Analyzer (IBM Instruments; Danbury, CT) with a standard three electrode arrangement (working: Pt gauze; auxiliary: Pt wire; reference: SCE). Oxidation was allowed to take place for 30 min for every 100 ml of reagent solution before use and the potential was held constant for the duration of the experiment. Continuous purging of the $Ru(bpy)_3^{3+}$ solution with helium eliminated oxide formation on the Pt electrodes and the reagent filter plus tubing [16]. The flow rate of the $Ru(bpy)_3^{3+}$ reagent stream was maintained at 0.7 ml min^{-1}. The carrier mobile phase pumped at 1.0 ml min^{-1} was acetonitrile–sodium acetate buffer containing 10 mM heptane–sulphuric acid (adjusted to pH 6.0) (27:73, v/v).

The erythromycin samples were prepared by first grinding and then weighing either about 0.15 g of the capsule or 0.50 g of the tablet. After dissolution by stirring for 20 min in 20 ml methanol, 10 ml water was added and the solutions filtered. The filtrate was collected in a 100-ml volumetric flask and brought to volume with water. Aliquots were taken to prepare approximately 20 $\mu g\ ml^{-1}$ solutions using 0.05 M phosphoric acid (pH 6.3) as the diluent. Standard samples were prepared in an acetonitrile–phosphate buffer (pH 6.3) (50:50, v/v). All solutions were stored in the refrigerator when not in use.

Results and Discussion

The optimum pH for the $Ru(bpy)_3^{3+}$ chemiluminescence reaction with erythromycin was found to be about 5.8 (Fig. 2) consistent with a previous study of trialkylamines [15]. Better reproducibility of the FIA signal was observed using a carrier stream with a pH of 6.0 with minimal sacrifice in the chemiluminescence signal. This is probably because erythromycin is most stable at a pH of 6–7. The response of erythromycin ethylsuccinate was equivalent to that of erythromycin.

The FIA peaks and corresponding linearity plot for samples ranging from 3 to 24 $\mu g\ ml^{-1}$ are shown in Fig. 3. The FIA peaks are sharp with little band broadening due to the fact that the connecting tube between the injector and detector was of small diameter (0.3 mm i.d.) and mixing took place in a low volume detector cell. Sampling throughout

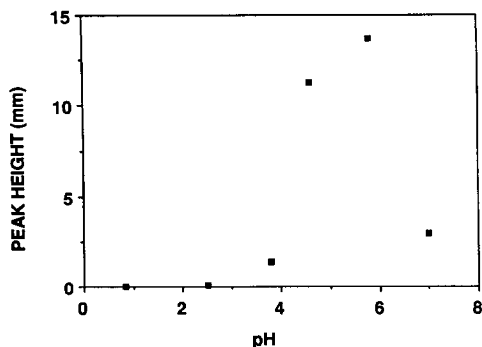

Figure 2
Chemiluminescent response for erythromycin as the free base (1 mM) as a function of pH. The carrier phase buffers were: 0.14 N H_2SO_4, pH 0.84; 10 mM NaH_2PO_4, pH 2.5; 10 mM acetate, pH 3.8; 10 mM acetate, pH 4.6; 10 mM acetate, pH 5.8; 10 mM Na_2HPO_4, pH 7.0. These data were taken using the Spiral-T cell detector designed in ref. [15].

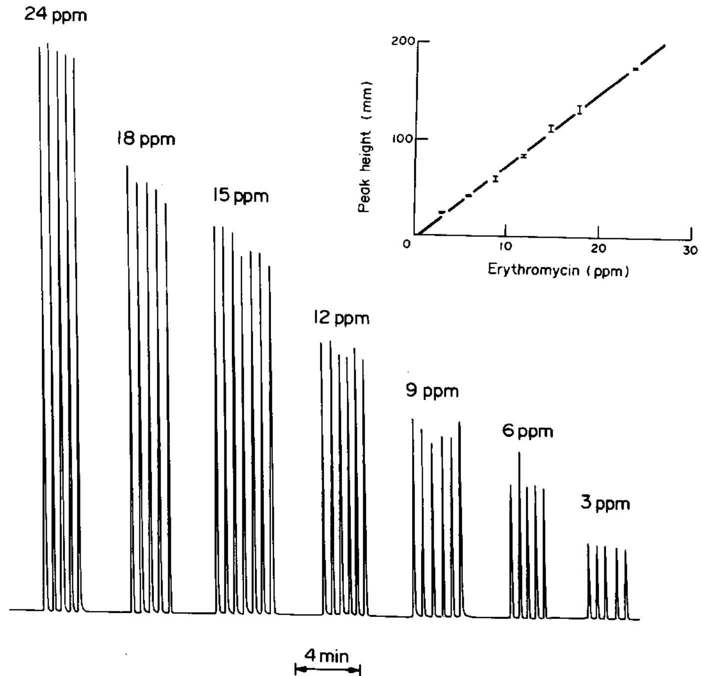

Figure 3
FIA peaks as a function of time (gain = 2) and calibration curve. Each point represents the average of five determinations and the error bars show standard deviations.

Table 1
Analysis of commercial erythromycin tablets and capsules

Sample	Manufacturer's specifications	Experimental values (n = 5)	Relative error (%)
Erythromycin ethylsuccinate tablets			
1	200 mg	210.3 ± 3.4	+4.8
2	200 mg	204.2 ± 2.4	+2.1
3	200 mg	192.4 ± 2.0	−4.0
Erythromycin capsule			
1	250 mg	251.2 ± 0.8	+0.5
2	250 mg	257.8 ± 1.8	+3.1

was estimated to be about 60 samples h^{-1} if an automatic injector was used. The linearity was good with a slope of 7.4 ± 0.2 and a correlation coefficient of 0.998. The relative standard deviation (RSD) of each point ranged from 0.7 to 4.6% with an average RSD value of 2.6%. The detection limit of 24 ng ml^{-1} corresponded to about 0.5 ng or 0.6 pmol injected. This value compared favourably to the electrochemical detection limits of 1–7 pmol [13, 14]. The recovery results for erythromycin ethylsuccinate tablets and erythromycin capsules are presented in Table 1. Interferences from either the tablet matrix or the orange dye present in part of the capsule were not a problem. The chemiluminescence method gave comparable data to the manufacturer's specification with an average relative error of +1% for the tablet and +1.8% for the capsule.

Analysis of these same samples using the sulphuric acid hydrolysis-colorimetric method [3] did not give accurate results. The capsule data were about 25% low and the tablet results were about three times too high. Clearly, the sample matrix presented difficulties; however, even with solid phase extraction, reproducible results were not obtained. It is expected that this chemiluminescence detection could be coupled with HPLC [16, 18] for the determination of erythromycin in more complicated matrices.

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