Flow-Injection Chemiluminescence Determination of Enalapril Maleate in Pharmaceuticals and Biological Fluids Using Tris(2,2′-bipyridyl)ruthenium(II)

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A chemiluminescence (CL) method using flow injection (FI) has been investigated for the rapid and sensitive determination of enalapril maleate. The method is based on the CL reaction of the drug with tris(2,2′-bipyridyl)ruthenium(II), $Ru(bipy)_3^{2+}$ and acidic potassium permanganate. After selecting the best operating parameters, calibration graphs were obtained over concentration ranges of 0.005 – 0.2 μg/ml and 0.7 – 100 μg/ml with a detection limit (S/N = 2) of 1.0 ng/ml. The average % found was 99.9 ± 0.7 and 100.2 ± 0.3 for the two concentration ranges respectively. %RSD (n = 10) for 5.0 μg/ml was 0.44. The method was successfully applied to the determination of enalapril maleate in dosage forms and biological fluids without interferences.

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Introduction

Enalapril maleate is a relatively new oral angiotensin-converting enzyme (ACE) inhibitor. Enalapril maleate, like its first-generation relative, captopril, has been shown to be effective in the treatment of hypertension and congestive heart failure.[1]

Enalapril [(*S*)-1-(*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl)-L-proline] is a prodrug which is de-esterified in the hepatic system to an active diacid form.[2] The drug is official in the USP 24.[3] The methods of analysis of the bulk drug and its tablets are high-performance liquid chromatography (HPLC) methods. It is also official in the European Pharmacopoeia[4] by a potentiometric titration method using sodium hydroxide.

Other than the published analytical profile,[5] several methods have been reported for the analysis of enalapril maleate either in pure form, in pharmaceutical preparations, in biological fluids, or in mixture with other drugs. These include spectrophotometric methods,[6-9] flow-injection spectrophotometric,[10] infrared method,[11] potentiometric selective membrane electrode methods,[12,13] NMR,[14] GC,[15] GCL/MS,[16] HPLC,[17-27] capillary electrophoresis,[28-31] radioenzymic[32] and radioimmunoassay.[33] Reviewing the literature revealed that up to the present time nothing has been published concerning the CL determination of enalapril maleate.

$Ru(bipy)_3^{2+}$ is an extremely versatile base reactant for a variety of CL processes, and has also recently become a useful CL reagent. The process involves the oxidation of $Ru(bipy)3^{2+}$ to $Ru(bipy)_3^{3+}$, which is then followed by reduction with an analyte species to produce the emission of light.[34] There have been a variety of methods employed to obtain the active oxidized reagent $Ru(bipy)_3^{3+}$. These include chemical, photochemical, electrochemical oxidation and *in situ* electrochemiluminescence. An analytical evaluation of commercially significant pharmaceutical drugs using $Ru(bipy)_3^{3+}$-CL detection has attracted considerable attention. A comprehensive review of the analytical applications of $Ru(bipy)_3^{3+}$ as a chemiluminescent reagent from 1978 to mid 1998 has been represented by Gerardi *et al*.[34]

The present paper describes the development of a simple flow-injection chemiluminometric method for the determination of enalapril maleate based on the chemical generation of $Ru(bipy)_3^{3+}$ by mixing two streams containing solutions of $Ru(bipy)_3^{2+}$ and acidic $KMnO_4$. The experimental conditions for the reaction were optimized, and the final procedure allowed the successful determination of the studied drug in pharmaceuticals and biological fluids.

Experimental

Apparatus and manifold

The flow system used for the determination and CL detection of enalapril maleate is shown schematically in Fig. 1. It consisted of a Gilson Minipuls 3MP4 peristaltic pump (two channels, variable speed) which was used to drive the carrier and the reagent streams through the flow system. Each stream was pumped at a constant flow rate using PTFE tubing (0.8 mm i.d.). The drug solution (250 μl) was injected through the sample injection valve, which allowed mixing of the sample with an acidified 1×10^{-3} mol/l $KMnO_4$ solution, and then combination with a 1×10^{-2} mol/l $Ru(bipy)_3^{2+}$ solution in a T-shaped piece before entering the flow cell. The flow cell was a coil made of 1.3 mm i.d. glass tubing spiralled to a diameter of 35 mm with five turns. The coiled glass flow cell was backed by a mirror and a sensitive photomultiplier tube (PMT, THORN EMI, 9789 QB) for measurement of the emitted light intensity. The PMT was operated at 1000 V, provided by a stable power supply (THORN EMI, Model PM28BN). The signal was

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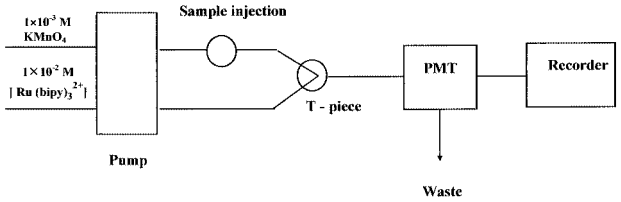

Fig. 1 Flow-injection manifold for CL determination of enalapril maleate.

recorded by a Yokogawa Model 3021 recorder (Yokogawa, Japan). The peak height was measured for each signal, and expressed as the voltage output of the PMT. The analytical signal was calculated as the sample output minus the blank output.

Reagents and materials

All of the reagents used were of analytical reagent grade, and the solutions were prepared with highly distilled water. Aqueous potassium permanganate (Fluka, UK) stock solution (1×10^{-3} mol/l) was prepared in 0.1 mol/l sulfuric acid (Riedel-de Haen). An aqueous (1×10^{-2} mol/l $Ru(bipy)_3^{2+}$ hexahydrate (Aldrich Chem. Co.), 0.1 mol/l hydrochloric acid (Riedel-de Haen), 0.1 mol/l and 5% sodium hydroxide (Winlab, UK) solutions were used. Other reagents used were chloroform (BDH, UK) and dichloromethane (Riedel-de Haen). Serum (Multi-serum-Normal, Randox Laboratories, UK) samples were obtained from commercial sources. Urine samples were obtained from healthy volunteers. Enalapril maleate was kindly provided by Merck (Rahway, USA). Dosage forms were obtained from local sources. A stock solution (1 mg/ml) of enalapril maleate was prepared in distilled water.

General procedure

The FI manifold described in Fig. 1 was used. A 250 µl drug solution was injected into a carrier stream of an acidified $KMnO_4$ solution, which was then combined with a stream of $Ru(bipy)_3^{2+}$ solution; the resulting peak height was measured. A calibration graph was constructed by plotting the peak height against the drug concentration.

Procedure for dosage forms

Ten tablets were weighed and pulverized. An accurately weighed amount of powder equivalent to 10 mg of the drug was dissolved in distilled water; it was sonicated for 10 min, then filtered in a 100 ml volumetric flask. The filtrate was diluted to volume with distilled water and proceeded as described above. The nominal content of the tablets was calculated either from a previously plotted calibration graph or by using the regression equation.

Procedure for spiked urine and serum

An aliquot of a standard aqueous solution of enalapril maleate containing 100 µg was added to 1 ml of urine or serum sample in a centrifuge tube and shaken well for 3 min. 1 ml of an aqueous solution of 0.1 mol/l NaOH was added, shaken and then 5 ml of dichloromethane were added. The mixture was vortex mixed at high speed for 5 min, and then centrifuged at 3000 rpm for 10 min. The resulting supernatant was transferred into a small conical flask. The extraction was repeated 2 times with 5 ml dichloromethane. The combined extracts were evaporated to dryness at room temperature and the residue was dissolved in 1 ml of 0.1 mol/l HCl. The solution was transferred into a 10 ml volumetric flask and completed to volume with distilled water, then analyzed according to the recommended procedure. The absolute recovery was determined by comparing the representative peak height of an extracted urine or serum sample with the peak height of the standard drug at the same concentration.

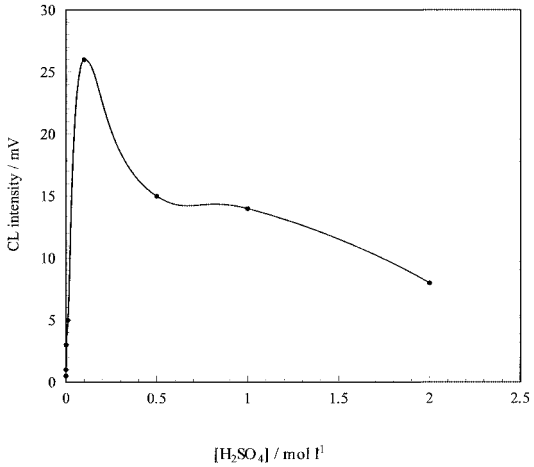

Fig. 2 Effect of the sulfuric acid concentration as a diluent for $KMnO_4$ on the CL intensity of enalapril maleate (1 µg/ml), $KMnO_4$ 1×10^{-3} mol/l, $Ru(bipy)_3^{2+}$ 1×10^{-2} mol/l, total flow rate 5.2 ml/min.

Results and Discussion

The flow injection chemiluminometric determination of enalapril maleate was studied using different oxidants to efficiently produce $Ru(bipy)_3^{3+}$ from $Ru(bipy)_3^{2+}$, such as potassium iodate, potassium bromate, potassium dichromate, potassium perchlorate, potassium persulfate, potassium permanganate, *N*-bromosuccinimide, cerium(IV) sulfate, sodium periodate and hydrogen peroxide, in an acidic or basic media. A very intense CL signal was obtained upon using $KMnO_4$ in an acidic medium and a very weak one on using acidic Ce(IV). Thus, $KMnO_4$ is used to produce $Ru(bipy)_3^{3+}$ that can be used for the determination of enalapril maleate by the FI technique.

Configuration designs

The FI configuration used for the determination of enalapril maleate was designed to provide different reaction conditions for magnifying the CL signal. The maximum CL intensity was obtained when the sample was injected into a stream of acidified $KMnO_4$, and then mixed with $Ru(bipy)_3^{2+}$ just before the detector.

Optimization of experimental variables

The optimum reaction conditions for enalapril maleate determination were investigated by injecting 250 µl of 1 µg/ml of the drug solution into the carrier stream at a flow rate of 2.6 ml/min in each channel. A series of experiments were conducted to establish the optimum analytical variables. The optimized parameters included the reagent concentrations and some manifold parameters including the total flow rate and the sample volume.

Effect of the sulfuric acid concentration on the CL intensity

The effect of the sulfuric acid concentration as a diluent for

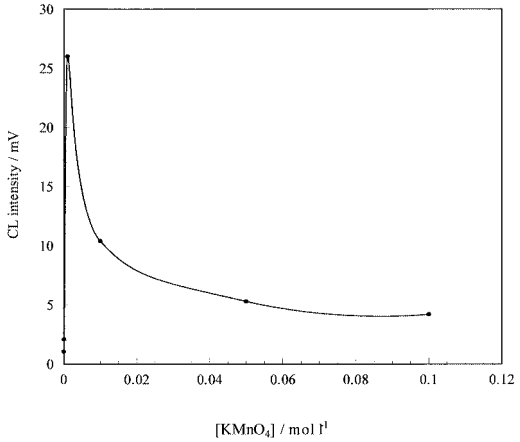

Fig. 3 Effect of the $KMnO_4$ concentration on the CL intensity of enalapril maleate (1 μg/ml), $Ru(bipy)_3^{2+}$ 1×10^{-2} mol/l, total flow rate 5.2 ml/min.

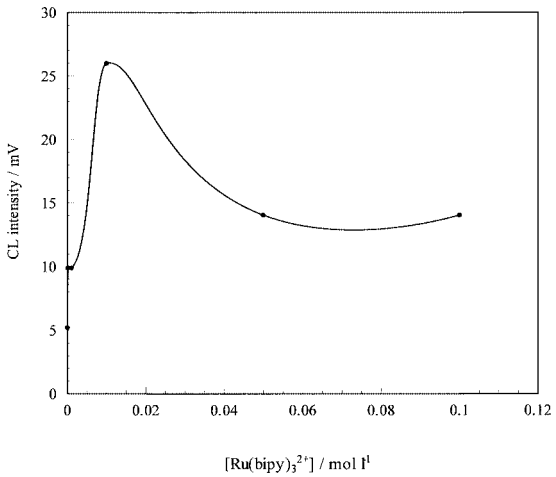

Fig. 4 Effect of the $Ru(bipy)_3^{2+}$ concentration on the CL intensity of enalapril maleate (1 μg/ml), $KMnO_4$ 1×10^{-3} mol/l, total flow rate 5.2 ml/min.

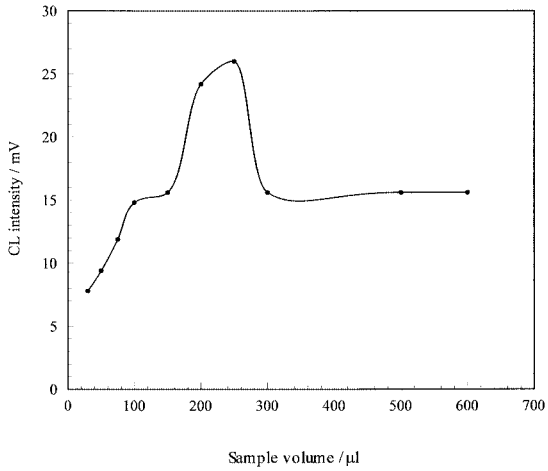

Fig. 5 Effect of the total flow rate on the CL intensity of enalapril maleate (1 μg/ml), $KMnO_4$ 1×10^{-3} mol/l, $Ru(bipy)_3^{2+}$ 1×10^{-2} mol/l.

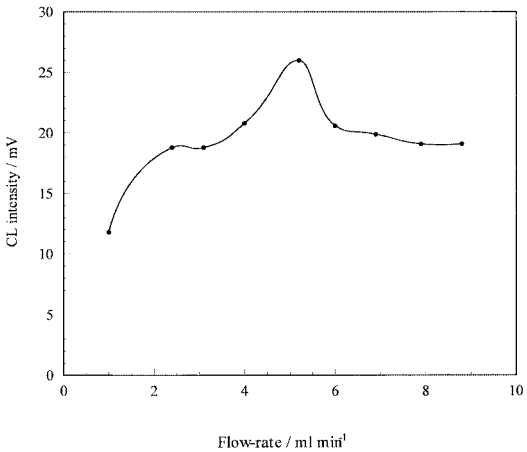

Fig. 6 Effect of the sample volume on the CL intensity of enalapril maleate (1 μg/ml), $KMnO_4$ 1×10^{-3} mol/l, $Ru(bipy)_3^{2+}$ 1×10^{-2} mol/l, total flow rate 5.2 ml/min.

$KMnO_4$ was studied in the range of 1×10^{-5} – 2.0 mol/l. Figure 2 shows this effect on the peak height. The greatest CL response was obtained with 0.1 mol/l H_2SO_4, above which the intensity was decreased.

Effect of the $KMnO_4$ concentration

A study was carried out in the range of 1×10^{-5} – 0.1 mol/l $KMnO_4$. The maximum intensity was obtained with 1×10^{-3} mol/l $KMnO_4$. Higher concentrations of $KMnO_4$ lowered the CL intensity, as shown in Fig. 3.

Effect of $Ru(bipy)_3^{2+}$ concentration

The concentration of $Ru(bipy)_3^{2+}$ concentration was studied in the range of 1×10^{-5} – 0.1 mol/l. Figure 4 shows that 1×10^{-2} mol/l gave the greatest CL intensity, above which the intensity decreased.

Effect of sensitizers

An investigation of the effect of sensitizers on the CL intensity using different fluorophores, such as quinine sulfate, Rhodamine 6G, Rhodamine B and fluoroescein showed that CL intensity, was not affected.

Effect of flow-rate

The total flow rates of the $Ru(bipy)_3^{2+}$ and $KMnO_4$ streams were varied over the range of 1.0 – 8.8 ml/min with equal flows in each channel. The obtained results show that 5.2 ml/min (2.6 ml/min for each channel) was the best total flow rate for the determination of the studied drug (Fig. 5).

Effect of the sample volume

The volume injected was varied between 30 and 600 μl. The resulting CL intensity increased with increasing volumes injected up to 250 μl, above which the intensity decreased and remained constant (Fig. 6).

Possible CL mechanism

$Ru(bipy)_3^{2+}$ CL has proven to be a very sensitive detection system for compounds which contain a secondary or tertiary aliphatic amine.[35] Enalapril maleate is a secondary amine. Thus, the proposed reaction mechanism is presumably similar to that reported previously[36] for amine determination utilizing its electrogenerated CL reaction with $Ru(bipy)_3^{2+}$. By analogy, the

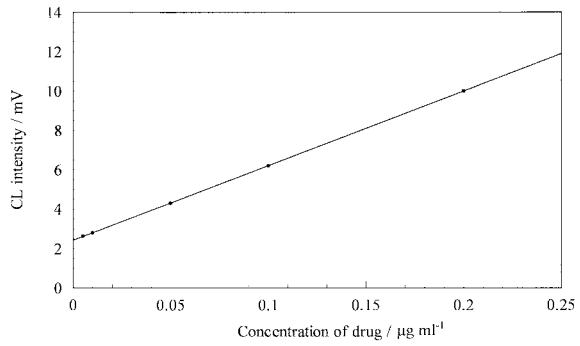

Fig. 7 Short-range calibration graph of enalapril maleate.

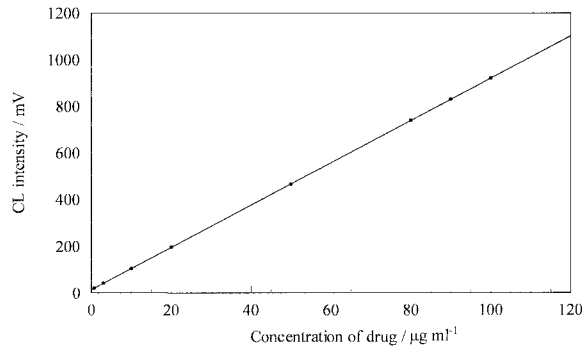

Fig. 8 Long-range calibration graph of enalapril maleate.

proposed mechanism involves the oxidation of $Ru(bipy)_3^{2+}$ and secondary amine present on enalapril maleate by $KMnO_4$. The oxidation product of amine undergoes deprotonation to form a radical. This reduces the $Ru(bipy)_3^{3+}$ to the excited state, which subsequently emits light.

Determination of enalapril maleate

Under the described experimental conditions, a series of standard solutions was pumped, each as 3 replicates, to test the linearity of the calibration graph. The CL intensity (I, mV) was linearly related to the enalapril maleate concentration over the ranges of 0.005 – 0.2 μg/ml and 0.7 – 100 μg/ml with a minimum detectability (S/N = 2) of 0.001 μg/ml. A linear-regression analysis of the results gave the following equation:
For the short-range concentration, (0.005 – 0.2) μg/ml,

$$I = 2.424 + 37.845C \quad (r = 0.99999, n = 5) \text{ (Fig. 7).}$$

The standard deviations of slope and intercept were 0.075 and 0.007, respectively.
For the long-range concentration, (0.7 – 100) μg/ml,

$$I = 14.123 + 9.063C \quad (r = 0.99999, n = 8) \text{ (Fig. 8).}$$

The standard deviations of slope and intercept were 0.007 and 0.395, respectively.

The precision of the method was evaluated by analyzing standard solutions of enalapril maleate. The results in Table 1 were in accord with those obtained by the official method.[4]

The reproducibility of the proposed method was checked with 10 samples of 5.0 μg/ml for the drug. The relative standard deviation (%RSD) value was 0.44, which illustrates that the results were highly reproducible.

Table 1 Analysis of some dosage forms containing enalapril maleate by the proposed and official methods

Preparation	Concentration taken/ μg ml^{-1}	Recovery, %	
		Proposed method	Official method[d]
Angiotec[a]	0.05	100.8	
(5 mg enalapril	0.1	99.7	
maleate/tablet)	1.0	99.9	
	10	100.0	
	40	100.1	
	70	100.8	
	90	100.8	
Mean ± S.D.		100.3 ± 0.5	99.4 ± 0.4
Renitec[b]	0.05	100.0	
(5 mg enalapril	0.1	99.9	
maleate/tablet)	1.0	99.9	
	10	99.9	
	40	100.01	
	70	100.0	
	90	100.9	
Mean ± S.D.		100.1 ± 0.4	100.2 ± 0.4
Enalapril tablets[c]	0.05	100.6	
(10 mg enalapril	0.1	100.1	
maleate/tablet)	1.0	100.0	
	10	100.4	
	40	100.1	
	70	100.1	
	90	100.8	
Mean ± S.D.		100.3 ± 0.3	100.6 ± 0.4

a. The Jordanian Pharm. Mtg. Co. Ltd.
b. Merck Sharp and Dohme B. V. Haarlem, Netherlands.
c. Product of SPIMACO.
d. Ref. 4.

Analysis of pharmaceutical preparations

The proposed method was successfully applied to the analysis of tablets containing enalapril maleate. The results in Table 1 agreed with those obtained by the official method.[4]

A statistical analysis[37] of these results using Student's t-test and the variance ratio F-test showed no significant difference between the performances of the methods as regards to the accuracy and precision.

Analysis of spiked urine and serum samples

The high sensitivity attained by the proposed method allows the determination of enalapril maleate in biological fluids. Enalapril, when administered orally, is rapidly absorbed and bioactivated extensively to enalaprilat. Following an oral administration of enalapril maleate to a person,[38] the peak serum concentrations of enalapril and enalaprilat occurred within 0.5 to 1.5 and 3 to 4 h, respectively. Based on urinary recovery of the total drug (enalapril plus its active metabolite, enalaprilat) absorption was at least 61%. The primary route of excretion of the drug was renal. Approximately 94% of the dose was recovered in the urine and feces as enalaprilat or enalapril.

Interference was expected from enalaprilat, since it contains secondary aliphatic amine. Thus, the separation of enalaprilat was necessary before determination of enalapril in the biological fluid. Plasma and urine were placed on XAD-4 columns, washed and then eluted with methanol to yield enalapril and enalaprilat.[39]

In the proposed method, extraction was required to avoid any interferences. The extraction process was carried out with dichloromethane after alkalinization with an aqueous solution of

Table 2 Determination of enalapril maleate in spiked urine and serum

Concentration taken/ μg ml^{-1}	% Found	
	Urine	Serum
0.05	99.6	99.8
0.1	99.7	100.1
1.0	99.6	100.4
10	100.3	99.7
40	100.0	100.3
70	99.7	100.8
90	100.6	100.8
Mean ± S.D.	99.9 ± 0.4	100.3 ± 0.4

sodium hydroxide. Excellent recoveries for urine and serum samples were obtained, as shown in Table 2.

Conclusion

A simple, rapid and highly sensitive chemiluminometric method is described for the determination of enalapril maleate in dosage forms and in biological fluids after extraction of both serum and urine to avoid possible interferences. It is more sensitive and rapid than most of the reported methods and characterized by instrumental simplicity, economy in the use of reagents and time efficiency. Solutions can be analyzed at a rate of 144 samples/h.

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