Research article

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Flow-injection chemiluminescence and electrogenerated chemiluminescence determination of escitalopram oxalate in tablet form

Nawal A. Alarfaj,* Fatma A. Aly and Abeer A. Al-Qahtany

ABSTRACT: Rapid, simple and highly sensitive flow-injection (FI) chemiluminescence (CL) and flow-injection electrogenerated chemiluminescence (ECL) methods were developed for the determination of escitalopram oxalate (ESC), a selective serotonin reuptake inhibitor used as an antidepressant drug. The CL method was based on the CL reaction of ESC with acidic cerium(IV) and tris (2,2′-bipyridyl)ruthenium(II) (Ru(*bipy*)$_3^{2+}$). Various experimental parameters affecting CL intensity were carefully studied and optimised. The method enabled the determination of 0.001-50 μg/mL of ESC in bulk form with a correlation coefficient r = 0.9999. The limit of detection (LOD) was 0.01 ng/mL (S/N = 3). The ECL method was based on the ECL reaction of Ru(*bipy*)$_3^{2+}$ with the drug in an acidic medium, permitting the determination of ESC in the range of 0.00001-70 μg/mL with r = 0.9999 and LOD of 1 x 10^{-4} ng/mL. The proposed methods were applied to the determination of ESC in commercial tablets. The results were compared statistically with those obtained from a published method using t- and F-tests. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: flow-injection chemiluminescence; flow-injection electrogenerated chemiluminescence; escitalopram oxalate; cerium(IV); tris(2,2′-bipyridyl) ruthenium(II); tablets

Introduction

Escitalopram is the pure S-(+)-enantiomer (single isomer) form of the racemic bicyclic phthalane derivative citalopram. Escitalopram oxalate (ESC) is designated as S-(+)-(1)-(3-(Dimethylamin0)propyl)-1-(*p*-fluorophenyl)-5-phthalancarbonitrile oxalate (1), which is shown in Figure 1. It is a selective serotonin reuptake inhibitor and belongs to a group of drugs known as antidepressants. These drugs help normalise levels of serotonin in the brain. Disturbances in the serotonin system of the brain are key factors in the development of depression and related disorders (1).

Several analytical methods have been reported for the determination of pure ESC in pharmaceuticals and biological fluids. Most reported methods are chromatography-based and no official method has been reported for the determination of ESC. The reported methods for analysis of ESC include UV–vis spectrophotometry (2–6), spectrofluorimetry (7,8), HPTLC (8–10), HPLC (3,11–13), LC/MS (13,14), LC/MS/MS (15) and capillary electrophoresis (16). The objective of this study was to develop new, simple and sensitive analytical procedures for the determination of pure ESC and ESC in certain pharmaceutical formulations.

Chemiluminescence (CL) and electrogenerated chemiluminescence (ECL) are powerful detection techniques for analytical determinations because of their very low detection limits, speed of analysis and wide linear working ranges that can be achieved using relatively simple instrumentation (17). One of the most interesting CL reactions involves the oxidation of tris(2,2′-bipyridyl) ruthenium(II) or Ru(*bipy*)$_3^{2+}$ to Ru(*bipy*)$_3^{3+}$, which is then reduced by an analyte species with a subsequent emission of light (18). However, Ru(*bipy*)$_3^{2+}$ ECL is observed when Ru(*bipy*)$_3^{2+}$ reacts with Ru(*bipy*)$_3^{+}$, yielding an excited state Ru(*bipy*)$_3^{2+}$*. ECL emission can also be obtained when a variety of oxidants and reductants react with the reduced or oxidised forms of Ru(*bipy*)$_3^{2+}$. Either the reductant or the oxidant can be treated as an analyte (19). This study describes the development of simple flow-injection chemiluminescence (FI-CL) and flow-injection electrogenerated chemiluminescence (FI-ECL) methods for the determination of escitalopram oxalate. The FI-CL method was based on the CL generated by the reaction of the drug with Ru(*bipy*)$_3^{2+}$ and cerric sulphate in a sulphuric acid medium. The FI-ECL method was based on the ECL generated by the reaction of the drug with Ru(*bipy*)$_3^{2+}$ in a sulphuric acid medium after electrolysis. To the best of our knowledge, no CL or ECL method has been reported for the determination of ESC. The proposed methods were satisfactorily applied to the determination of the studied drug in tablets.

Experimental

Instrumentation and manifold for various methods

CL method. The flow system used for the determination and CL detection of escitalopram oxalate is shown schematically in Figure 2A. CL measurements were made with a flow-injection CL analyser that featured two basic components; a detector housing and a flow-through system that allowed mixing of the

* Correspondence to: Nawal A. Alarfaj. Department of Chemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia. Tel. & Fax: +96612914322. E-mail: nalarfaj@hotmail.com

Department of Chemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia

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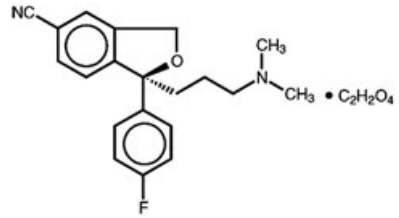

Figure 1. Chemical structure of escitalopram oxalate.

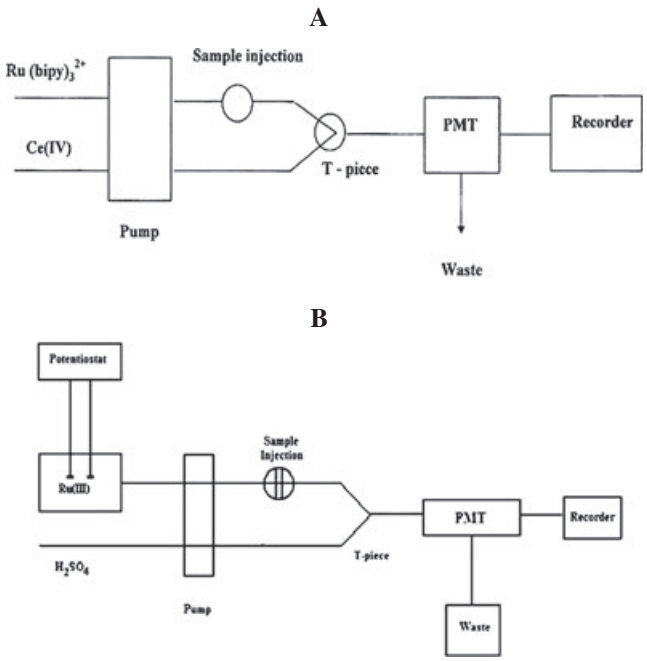

Figure 2. A: Flow injection manifold for CL determination of escitalopram oxalate. The carrier was 1 x 10^{-3} M Ru$(bipy)_3^{2+}$ and the oxidant was 1 x 10^{-3} M Ce(IV). PMT = photomultiplier tube. B: Electrogenerated flow injection manifold for ECL determination of escitalopram oxalate. The carrier was 4 x 10^{-3} M Ru$(bipy)_3^{2+}$ after electrolysis and (H_2SO_4) = 1 x 10^{-4} M.

sample with the Ru$(bipy)_3^{2+}$ solution, which was then combined with the Ce(IV) solution just before the sample reached the detector.

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, enabling the flowing emitting solution to remain in view of the detector. The coiled glass flow cell was backed by a mirror for maximum light collection and a sensitive photomultiplier tube (PMT, model Thorn EMI, 9789 QB, UK) for measurement of the emitted light intensity. The PMT was operated at 1000 V provided by a stable high voltage power supply (Thor EMI, Model PM 28BN). No wavelength selection was involved.

A 2-channel peristaltic pump (Gilson MiniPuls 3MP4) was used to deliver the reagents. A solenoid-activated rotary valve (Rheodyne 5020) was used to inject the drug solution through a carrier stream of polytrifluoroethylene) (PTFE) tubing of 0.06 mm i.d., which was used throughout the remainder of the manifold. A strip-chart recorder (type 41, Chessel, Ltd. Bournemouth, UK,) was fed to the PMT and peak heights were measured manually. The drug solution (150 µL) was injected through the sample injection valve, which allowed the mixing of the sample with 1 x 10^{-3} M Ru$(bipy)_3^{2+}$ solution, and then combined with an acidified 1 x 10^{-3} M Ce(IV) solution just before reaching the detector. The emitted intensity was measured by the PMT and the signal was recorded by the recorder. Peak height was measured for each signal and expressed as a voltage output of the photomultiplier tube.

ECL method. The same instrument was used as described above but an electrode control circuit (Model 3006) was added to the system. Potentials were applied to the electrodes using a two-electrode potentiostat. Two similar platinum electrodes were used. A magnetic stirrer (3451, Snijders, Netherland) was used during electrolysis. A two-line manifold was used for ECL determination of ESC (Fig. 2B). The drug solution (150 µL) was injected into a stream of acidified 4 x 10^{-3} M Ru$(bipy)_3^{2+}$ after electrolysis and then mixed with a stream of 1 x 10^{-4} M H_2SO_4 solution just before reaching the detector, and the resulting peak heights were measured.

Reagents and materials

All reagents were of analytical reagent grade and the solutions were prepared with distilled water. A stock solution of 1 x 10^{-3} M cerric ammonium sulphate dihydrate (Fluka, Switzerland) was prepared in 0.1 M sulphuric acid (BDH Ltd., Poole, UK). A stock solution of 1 x 10^{-3} M aqueous Ru$(bipy)_3^{2+}$ (Sigma-Aldrich, St. Louis, MO, USA) was prepared by dissolving ruthenium dipyridyl chloride hexahydrate in distilled water. Acidified 4 x 10^{-3} M Ru$(bipy)_3^{2+}$ was prepared in 0.1 M H_2SO_4, and an aqueous solution of 1 x 10^{-4} M H_2SO_4 was also prepared. Pure escitalopram oxalate was kindly supplied by Saudi Pharmaceutical Industries and Medical Appliances Corporation (SPIMACO, Saudi Arabia), Al-Qassim Pharmaceutical Plant. Cipralex® tablets (Batch No. 2175341) containing 10 mg escitalopram were manufactured by H. Lundbeck A/S (Valby, Denmark) and obtained from commercial sources.

Preparation of standard solutions

A standard stock solution (0.1 mg/mL) of escitalopram oxalate was prepared in distilled water. This solution was found to be stable for at least one week without alteration when stored in the refrigerator. Working standard solutions were prepared from the stock solution by further dilution with distilled water immediately before use.

General procedures

CL method. Working standard solutions of ESC were prepared in distilled water from the stock solution in the range of 0.001-50 µg/mL. A 150-µL portion was injected into a stream of 1 x 10^{-3} M Ru$(bipy)_3^{2+}$ solution, which then was combined with a stream of 1 x 10^{-3} M Ce(IV) solution, and the resulting peak heights were measured. Calibration curves were prepared by plotting the peak heights as CL intensity (mV) against the drug concentrations. Additionally, the regression equations were derived.

ECL method. Working standard solutions of ESC were prepared in distilled water from the stock solution in the range of 1 x 10^{-5}-70 µg/mL. A 150-µL portion was injected into a stream of acidified 4 x 10^{-3} M Ru$(bipy)_3^{2+}$ after electrolysis, which was then combined with a stream of 1 x 10^{-4} M H_2SO_4 solution, and the

resulting peak heights were measured. Calibration curves were prepared by plotting the ECL intensities (mV) against the drug concentrations. In addition, the corresponding regression equations were calculated.

Tablets

An accurately weighed amount of 10 powdered tablets equivalent to 10.0 mg of escitalopram (12.77 mg escitalopram oxalate) was dissolved in 60 mL of distilled water and sonicated for 20 min before being filtered into a 100-mL volumetric flask. The filtrate was diluted with distilled water to the mark and the tablet solution was analysed as described above using a general procedure for CL and ECL methods. The nominal content was calculated either from the previously plotted calibration curves or by using corresponding regression equations.

Results and discussion

FI-CL method

The reproducibility, selectivity and speed of signal detection in CL analysis can be highly improved through combination with the FI technique (20–24). As previously reported (25–28), Ru(*bipy*)$_3^{3+}$ was obtained from Ru(*bipy*)$_3^{2+}$, which becomes a useful CL reagent and utilises various oxidants. In the present study, a very weak CL signal was obtained with potassium permanganate. Other oxidants such as hydrogen peroxide, N-bromosuccinimide, potassium dichromate and potassium bromate emitted no CL signals. Maximum CL intensity was obtained when cerric ammonium sulphate dihydrate was used as an oxidant in an acidic medium, which was then used to oxidise Ru(*bipy*)$_3^{2+}$ to Ru(*bipy*)$_3^{3+}$, and the latter was then reduced by ESC with the subsequent emission of light. The maximum drug CL signal was obtained when the drug sample was injected into a stream of 1 x 10^{-3} M Ru(*bipy*)$_3^{2+}$ and then mixed with 1 x 10^{-3} M Ce(IV) prior to detection. Various experimental parameters affecting CL intensity were studied.

Configuration designs for FI-CL method

A two-line FI manifold was used for the chemiluminometric determination of ESC, which was designed to provide different reaction conditions for magnifying the CL signal generated by the reaction. The CL signal was obtained only when the sample was injected into a stream of Ru(*bipy*)$_3^{2+}$ and then mixed with Ce(IV) prior to the detector (Fig. 2A).

Optimisation of experimental variables for FI-CL Method

The effects of various experimental conditions on CL intensity were studied. A series of experiments were conducted to establish the optimum analytical variables by injecting 150 µL 1.0 µg/mL of the drug solution into the carrier stream. Optimised parameters included reagent concentrations and certain manifold parameters, including flow rate, sample volume and reaction coil length from the T-piece to the PMT. These variables were adjusted using the univariate optimisation procedure by changing one variable at each step and keeping the others at their optimum values.

Effect of sulphuric acid concentration as a solvent for Ce(IV)

Cerric ammonium sulphate is not readily soluble in water but becomes stable when dissolved in sulphuric acid solution. Therefore, the effect of sulphuric acid concentration on the CL emission for ESC was studied in the range from 1 x 10^{-2} to 1.0 M sulphuric acid. The highest CL signal was obtained with 0.1 M sulphuric acid. Thus, this concentration was used in the preparation of Ce(IV) solutions for ESC determination.

Effect of cerium(IV) concentration

The effect of Ce(IV) concentration on the CL intensity of ESC was studied in the range of 1 x 10^{-4} to 5 x 10^{-2} M Ce(IV) (Fig. 3). Maximum CL signal was obtained with 1 x 10^{-3} M Ce(IV), which was used for further investigation.

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

The dependence of the CL signal on the concentration of Ru(*bipy*)$_3^{2+}$ was examined in the range of 1 x 10^{-4} to 5 x 10^{-2} M Ru(*bipy*)$_3^{2+}$. Figure 4A shows the effect of Ru(*bipy*)$_3^{2+}$ concentration on the CL intensity of ESC. CL intensity increased with increasing

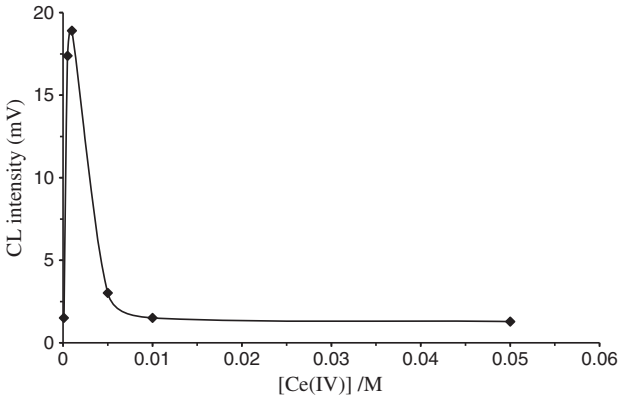

Figure 3. Effect of Ce(IV) concentration on the CL intensity of escitalopram oxalate. The injected drug solution was 1.0 µg/mL, (Ru(*bipy*)$_3^{2+}$) = 1 x 10^{-3} M, loop size was 150 µL and total flow rate was 12 mL/min.

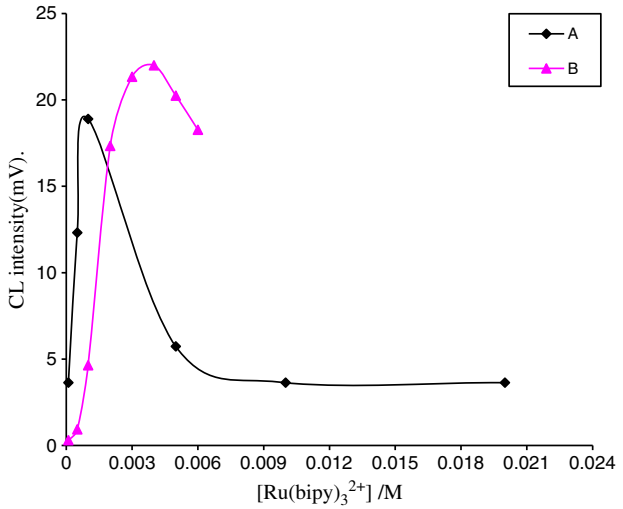

Figure 4. A: Effect of Ru(*bipy*)$_3^{2+}$ concentration on CL intensity of escitalopram oxalate. The injected drug solution was 1.0 µg/mL, (Ce(IV)) = 1 x 10^{-3} M, loop size was 150 µL, and total flow rate was 12 mL/min. B: Effect of Ru(*bipy*)$_3^{2+}$ concentration on ECL intensity of escitalopram oxalate. The injected drug solution was 1.0 µg/mL, (H_2SO_4) = 1 x 10^{-4} M, loop size was 150 µL and total flow rate was 7.5 mL/min.

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$Ru(bipy)_3^{2+}$ concentrations up to 1 x 10^{-3} M, after which the intensity began decreasing. Therefore, 1 x 10^{-3} M $Ru(bipy)_3^{2+}$ was used for further studies.

Effect of total flow rate

Once the concentrations of reagents were optimised, the effect of the flow rate was studied. The solutions of Ce(IV) and Ru $(bipy)_3^{2+}$ were introduced into the manifold at equal flow rates. The effect of the total flow rate on CL intensity is shown in Figure 5A. Optimum CL intensity was obtained using a total flow rate of 12 mL/min (i.e. 6 mL/min in each channel). Higher flow rates produced only a minor increase in CL intensity but consumed more reagents.

Effect of sample volume

The variation in the CL intensity with the injected sample volume was studied in the range of 30–600 μL by changing the length of the sample loop connected to the injection valve. Peak height increased with increasing sample volumes up to 150 μL of ESC as shown in Figure 6A.

Effect of reaction coil length

The effect of the reaction coil length from the T-piece to the PMT was studied in the range of 10–200 cm. Maximum CL intensity was obtained using 10 cm of coil length, which was a suitable coil length for this study.

FI-ECL Method

Ru $(bipy)_3^{2+}$ has been the most widely studied ECL reagent because of its chemical stability, redox properties, excited state reactivity, luminescence emission and excited state life (29). In FI-ECL, the ECL is generated by the reaction of ESC with acidified $Ru(bipy)_3^{2+}$ after electrolysis.

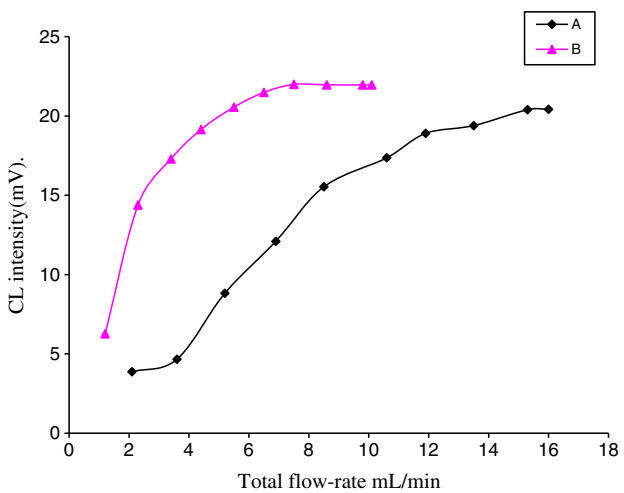

Figure 5. A: Effect of total flow rate on CL intensity of escitalopram oxalate. The injected drug solution was 1.0 μg/mL, Ce(IV) = 1 x 10^{-3} M, ($Ru(bipy)_3^{2+}$) = 1 x 10^{-3} M and loop size was 150 μL. B: Effect of total flow rate on ECL intensity of ESC. The injected drug solution was 1.0 μg mL^{-1}, $Ru(bipy)_3^{2+}$ = 4 x 10^{-3} M, (H_2SO_4) = 1 x 10^{-4} M and loop size was 150 μL.

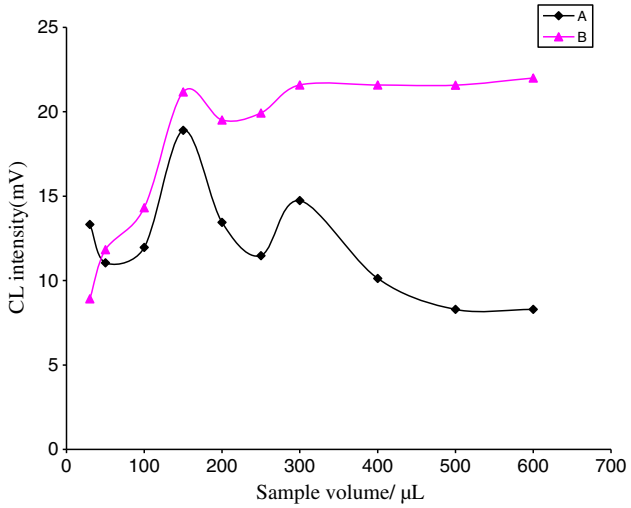

Figure 6. A: Effect of sample volume on CL intensity of escitalopram oxalate. The injected drug solution was 1.0 μg/mL, Ce (IV) = 1x 10^{-3} M, $Ru(bipy)_3^{2+}$ = 1 x 10^{-3} M and total flow rate was 12 mL/min. B: Effect of sample volume on ECL intensity of escitalopram oxalate. The injected drug solution was 1.0 μg/mL, $Ru(bipy)_3^{2+}$ = 4 x 10^{-3} M, H_2SO_4 = 1 x 10^{-4} M and total flow rate was 7.5 mL/min.

Configuration designs of the FI-ECL method

A two-line manifold was used for ECL determination of ESC. Maximum ECL intensity was obtained when the sample was injected into a stream of acidified 4 x 10^{-4} M $Ru(bipy)_3^{2+}$ after electrolysis for 70 min and then mixed with 1 x 10^{-4} M H_2SO_4 prior to detection. A schematic diagram of the manifold is shown in Fig. 2B.

Optimisation of experimental variables of the FI-ECL method

The optimisation of chemical variables included reagent concentrations and certain physical variables, including flow rate, sample volume and reaction coil length from the T-piece to the PMT. Since the principle used for measuring the drug concentration with FI-ECL depends on the oxidation of Ru $(bipy)_3^{2+}$ to Ru $(bipy)_3^{3+}$, it is therefore essential to study the effect of the reagent oxidation time. These variables were performed using the univariate optimisation procedure by changing one variable at each step and keeping the others at their optimum values.

Effect of oxidation potential of Ru $(bipy)_3^{2+}$. The effect of the oxidation potential of Ru $(bipy)_3^{2+}$ on ECL intensity using 1.0 μg/mL of ESC was studied in the range of 0.4 to 3.0 V. ECL intensity, measured as an average peak height, was plotted versus the applied voltage. Each point was the mean of three replicate injections. Optimum voltage was found to be 2.2 V. This voltage was used throughout the remainder of the study.

Effect of oxidation time of Ru $(bipy)_3^{2+}$. The effect of oxidation time of Ru $(bipy)_3^{2+}$ on ECL intensity using 1.0 μg/mL of ESC was studied in the range of 10–110 min. Maximum ECL signal was obtained after 70 min of oxidation. The long oxidation time was due to the polar areas of the two small plates of platinum used.

Effect of Ru $(bipy)_3^{2+}$ concentration. The concentration of Ru $(bipy)_3^{2+}$ was studied in the range of 1 x 10^{-4} - 6 x 10^{-3} M. Figure 4B shows that 4 x 10^{-3} M gave the greatest ECL intensity

and higher concentrations of Ru$(bipy)_3^{2+}$ lowered ECL intensity. Therefore, 4 x 10^{-3} M Ru$(bipy)_3^{2+}$ was used for further investigation.

Effect of sulphuric acid concentration as a solvent for Ru $(bipy)_3^{2+}$. A very weak ECL signal appeared when Ru$(bipy)_3^{2+}$ was dissolved in distilled water. This signal significantly increased when Ru$(bipy)_3^{2+}$ was dissolved in H_2SO_4. A maximum ECL signal was obtained when the sample was injected into a stream of acidic solution of 4 x 10^{-3} M Ru$(bipy)_3^{2+}$ after electrolysis and then mixed with 1 x 10^{-4} M H_2SO_4 prior to reaching the detector. The effect of H_2SO_4 concentration as a solvent of Ru$(bipy)_3^{2+}$ on ECL intensity of ESC was studied in the range of 0.01 - 1.0 M H_2SO_4. The highest ECL emission was obtained when Ru $(bipy)_3^{2+}$ was dissolved in 0.1 M sulphuric acid, which was used for further investigation.

Effect of sulphuric acid reagent concentration. The effect of H_2SO_4 concentration as a reagent flowing in line 2 of the manifold (Fig. 2B) was studied in the range of 1 x 10^{-5} - 0.01 M H_2SO_4. The greatest ECL intensity was obtained with 1 x 10^{-4} M H_2SO_4, above which intensity was nearly constant. Therefore, 1 x 10^{-4} M H_2SO_4 was used for further studies.

Effect of total flow rate. The total flow rate of the reagents solutions was optimised to obtain satisfactory ECL intensity. The effect of total flow rate was studied by keeping all other conditions constant over the range of 1.2 - 10.1 mL/min and using equal flows in each channel. The results showed that 7.5 mL/min (3.75 mL/min for each channel) was the best flow rate for ECL of ESC (Fig. 5B).

Effect of sample volume. The injected sample volume was varied from 30–600 μL by changing the size of the sample loop connected to the injection valve. The best sample volume for ESC was 150 μL as shown in Figure 6B.

Effect of reaction coil length. Maximum ECL signal was obtained using 10 cm as the optimal reaction coil length, measured from the T-piece to the PMT. At reaction coil lengths longer than 10 cm, intensity began to decrease.

Determination of escitalopram oxalate by the proposed methods

Under the optimum conditions mentioned above, a series of working ESC solutions were each injected in triplicate. Plots of CL and ECL intensity (mV) versus drug concentration were found to be linear over the ranges of 0.001-50 μg/mL and 1 x 10^{-5}-70 μg/mL of ESC, respectively. Linear regression analysis of these data is shown in Table 1.

Application of the proposed methods

To evaluate the analytical usefulness of the proposed CL and ECL methods, ESC was determined in a commercially available pharmaceutical product (Ciprolex tablets). The recoveries of different concentrations of ESC were based on the average of three replicate determinations. The results shown in Table 2 were in good agreement with those obtained by the published spectrophotometric method (3). Statistical analysis (30) of the results from the proposed and comparison methods showed no significant difference between them with regard to accuracy (t-test) and precision (F-test) as illustrated in Table 3.

Validity of the proposed methods

Linearity and range. The calibration curves for the determination of ESC by the proposed methods were constructed by plotting CL or ECL intensity versus the concentration of ESC. The curves were found to be rectilinear over the concentration ranges cited in Table 1. Regression analysis of the data using the method of least squares gave high values of the correlation coefficients (r) and small values of standard deviations of the intercepts (δ_a) and standard deviations of the slopes (δ_b). These data proved the linearity of the calibration curves (Table 1).

LOD and limit of quantitation (LOQ). LOD and LOQ were determined according to ICH (31) guidelines. LOD was determined by establishing the minimum level at which the analyte could reliably be detected (i.e. a signal-to-noise ratio of 3:1), while LOQ was determined by establishing the lowest concentration of the analyte that could be determined with acceptable precision and accuracy (i.e. a signal-to-noise ratio of 10:1). Results of both CL and ECL methods are shown in Table 1.

Table 1. Analytical performance data for the FI-CL and FI-ECL determination of escitalopram oxalate.

Parameter	FI-CL value		FI-ECL value	
	Short range	Long range	Short range	Long range
Linear range (μg/mL)	0.001-1.0	1.0-50	1 x 10^{-5}-0.1	0.5-70
Regression equation:	I = 0.1137	I = −0.1009	I = 0.1602	I = 3.0609
$I^a = a + b\ C$	+ 8.784C	+ 20.03C	+ 30.473C	+ 21.373C
Correlation	0.9999	0.9999	0.9999	0.9999
coefficient (r)	(*n* = 7)	(*n* = 6)	(*n* = 8)	(*n* = 9)
δ_a*: standard deviation of intercept*	0.002	1.700	0.003	2.305
δ_b*: standard deviation of slope*	0.005	0.057	0.08	0.058
LOD[b] *(μg/mL)*	1 x 10^{-5}		1 x 10^{-7}	
LOQ (μg/mL)	0.001		1 x 10^{-5}	

[a]Intensity (mV)
[b]S/N = 3

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Table 2. Analysis of escitalopram oxalate in commercial tablets by the proposed FI-CL, FI-ECL and the published spectrophotometric methods.

Preparation	FI-CL method		FI-ECL method		Comparison method (3)
	Concentration taken (μg/mL)	% Found[a]	Concentration taken (μg/mL)	% Found[a]	% Found
Cipralex® tablets (10 mg/tablet)[b]	0.001	98.33	0.01	98.68	
	0.01	100.00	0.1	100.02	
	1.00	101.06	1.0	101.61	
	20.0	101.13	10.0	98.25	
	30.0	100.15	30.0	100.24	
	50.0	98.16	60.0	99.77	
Mean ± S.D.	99.81 ± 1.294		99.76 ± 1.197		99.66 ± 1.152
Student's t-value	0.169 (2.365)[c]		0.119 (2.365)[c]		
Variance ratio F-value	1.262 (19.30)[d]		1.079 (19.30)[d]		

[a]Each results is the average of three separate determinations
[b]Products of H. Lundbeck A/S, Denmark.
[c]Tabulated t-value at confidence level 95% (30)
[d]Tabulated F-value at confidence level 95% (30)

Table 3. Analysis of escitalopram oxalate in pure form by the proposed FI-CL, FI-ECL and the published spectrophotometric methods.

	FI-CL method				FI-ECL method				Published method (3)
Concentration taken (μg/mL)	Concentration found(μg/mL)	% Error[a]	% Found[b]	Concentration taken(μg/mL)	Concentration found(μg/mL)	% Error[a]	% Found[b]	% Found[b]
0.01	0.009	−0.82	99.18	$1x10^{-5}$	0.98×10^{-5}	−1.55	98.45	
0.10	0.100	0.42	100.42	0.10	0.099	−0.25	99.75	
1.10	1.000	0.01	100.01	20.0	19.98	−0.12	99.88	
10.0	9.990	−0.09	99.90	30.0	30.03	0.12	100.12	
30.0	30.21	0.69	100.69	60.0	59.93	−0.11	99.89	
50.0	49.94	−0.14	99.86	70.0	69.71	−0.41	99.59	
Mean ± S.D.		100.01 ± 0.52				99.61 ± 0.60		99.94 ±0.33
Student's t-value		0.209 (2.365)[d]				0.873 (2.365)[d]		
Variance ratio F-value		2.444 (1/9.30)[e]				3.207 (19.30)[e]		

[a]Calculated as ((measured value-true value)/(true value)) x 100
[b]Each result is the average of three separate determinations.
[c]Mean ± S.D. for three different concentrations.
[d]Tabulated t-value at confidence level 95% (30)
[e]Tabulated F-value at confidence level 95% (30)

Accuracy and precision. To prove the accuracy of the proposed methods, the results of the pure ESC assay were compared to those obtained using the published spectrophotometric method (3). Statistical analysis (30) of the results obtained from the proposed and published methods using Student's t-test and variance ratio F-test showed no significant difference between the two methods with regard to accuracy and precision (Table 3). Intraday precision of the two methods was assessed using three concentrations from both short- and long-range concentrations of the drug with three replicates of each concentration. Interday precision was determined by triplicate analyses of the concentrations of the drug over three consecutive days. Standard deviations were found to be very small, indicating reasonable repeatability and high precision of the methods (Table 4).

Selectivity. The selectivity of the optimised procedures for ESC assay was examined in the presence of the excipients found in Cipralex tablets: talc, croscarmellose sodium, microcrystalline cellulose, colloidal silicon dioxide and magnesium stearate. The film coating contained hydroxypropylmethyl cellulose, titanium dioxide and polyethylene glycol. It was found that there was no significant interference from the excipients. Accordingly, the proposed methods were considered to be selective.

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Table 4. Precision data for the determination of escitalopram oxalate by the proposed FI-CL and FI-ECL methods.

FI-CL method									FI-ECL method			
Concentration (μg/mL)									Concentration (μg/mL)			
Intraday	0.01	0.10	1.00	10.0	30.0	50.0	0.005.	0.01	0.05	10.0	40.0	70.0
			% Found[a]						% Found[a]			
	98.65	99.35	100.01	99.90	99.66	99.56	99.32	100.27	101.72	101.03	100.82	99.45
	99.18	100.42	99.90	98.80	99.45	100.06	98.97	98.39	100.79	100.56	100.00	99.25
	99.71	100.42	100.54	99.90	100.51	99.96	100.49	99.38	100.66	100.09	99.88	99.78
			Mean						Mean			
	99.18	100.06	100.15	99.53	99.87	99.86	99.26	99.35	101.05	100.56	100.23	99.49
			S.D.						S.D.			
	0.530	0.617	0.342	0.635	0.561	0.264	1.113	0.940	0.578	0.470	0.511	0.268
			S (mean)[b]						S (mean)[b]			
	0.306	0.356	0.197	0.366	0.324	0.153	0.642	0.543	0.334	0.271	0.295	0.155
Interday			% Found[a]						% Found[a]			
	99.18	99.88	99.90	100.39	99.55	99.76	99.63	99.69	100.04	100.55	99.65	99.39
	100.77	100.95	101.07	99.40	100.08	99.40	98.32	100.02	101.06	100.79	100.59	99.12
	100.24	101.48	100.01	100.89	100.93	100.05	100.28	98.71	99.87	99.93	99.73	99.98
			Mean						Mean			
	100.06	100.72	100.33	100.23	100.19	99.74	99.41	99.47	100.32	100.42	99.99	99.49
			S.D.						S.D.			
	0.809	0.815	0.646	0.758	0.696	0.325	0.998	0.681	0.643	0.444	0.521	0.439
			S (mean)[b]						S (mean)[b]			
	0.467	0.470	0.373	0.438	0.402	0.188	0.576	0.393	0.371	0.256	0.301	0.253

[a]Each result is the average of three separate experiments.
[b]Calculated as (S.D./$\sqrt{n}$)

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Ruggedness. To examine the ruggedness of the method, the intraday and interday precisions were evaluated as shown in Table 4. Precision of the methods was found to be fairly high as indicated by the low S.D. values.

Mechanism of the proposed methods

CL mechanism. $Ru(bipy)_3^{2+}$ CL has proven to be a very sensitive detection system for compounds that contain a secondary or tertiary aliphatic amine (32). ESC in the current study contained a tertiary amine; thus, the reaction mechanism was presumably similar to that reported previously for amine determination using an electrogenerated CL reaction with $Ru(bipy)_3^{2+}$ (32,33). By analogy, the proposed mechanism involved the oxidation of $Ru(bipy)_3^{2+}$ and the tertiary amine present in ESC by Ce(IV). The oxidation product of the amine underwent deprotonation to form a radical. This reduced the $Ru(bipy)_3^{3+}$ to the excited state, which subsequently emitted light as shown below:

$$Ru(bipy)_3^{2+} + Ce(IV) \rightarrow Ru(bipy)_3^{3+} + Ce(III)$$
$$\text{escitalopram oxalate} + Ce(IV) \rightarrow \text{escitalopram oxalate}^{+} + Ce(III)$$
$$\text{escitalopram oxalate}^{\cdot +} \rightarrow \text{escitalopram oxalate}^{\cdot} + H^{+}$$
$$Ru(bipy)_3^{3+} + \text{escitalopram oxalate} + H_2O \rightarrow (Ru(bipy)_3^{2+})^* + \text{escitalopram oxalate fragment} + H^{+}$$
$$(Ru(bipy)_3^{2+})^* \rightarrow Ru(bipy)_3^{2+} + h\nu$$

ECL mechanism. The reaction mechanism involved the reaction of ESC as a reductant of $Ru(bipy)_3^{3+}$ to form $Ru(bipy)_3^{+}$. Then, the excited species $(Ru(bipy)_3^{2+})^*$ was formed by the reaction of $Ru(bipy)_3^{+}$ and $Ru(bipy)_3^{3+}$. This mechanism was similar to that reported by Lee (19).

$$Ru(bipy)_3^{2+} - e^{-} \rightarrow Ru(bipy)_3^{3+}$$
$$Ru(bipy)_3^{3+} + \text{escitalopram oxalate} \rightarrow Ru(bipy)_3^{+}$$
$$Ru(bipy)_3^{+} + Ru(bipy)_3^{3+} \rightarrow Ru(bipy)_3^{2+*} + Ru(bipy)_3^{2+}$$
$$Ru(bipy)_3^{2+*} \rightarrow Ru(bipy)_3^{2+} + h\nu$$

Conclusions

Simple, rapid and highly sensitive FI-CL and FI-ECL methods were described for the determination of escitalopram oxalate, a selective serotonin reuptake inhibitor used as an antidepressant drug. The developed methods were applied with good accuracy and precision to quantify the drug in tablet form. The proposed FI-ECL method was more sensitive than the FI-CL method and therefore more economical because fewer reagents were consumed. Both of the methods were accurate, precise, rapid and more sensitive than most reported methods, with the advantages of low running and instrumentation costs, easy-to-handle reagents, low reagent consumption and minimal waste production. Solutions were analysed at a rate of 200 and 164 samples/h using FI-CL and FI-ECL methods, respectively.

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