Flow-injection chemiluminescent determination of metoclopramide hydrochloride in pharmaceutical formulations and biological fluids using the $[Ru(dipy)_3^{2+}]$–permanganate system

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Abstract

A flow-injection (FI) methodology using (2,2′-dipyridyl) ruthenium(II) $[Ru(dipy)_3^{2+}]$ chemiluminescence (CL) was developed for the rapid and sensitive determination of metoclopramide hydrochloride. The method is based on the CL reaction of metoclopramide with $Ru(dipy)_3^{2+}$ and $KMnO_4$ in a sulfuric acid medium. Under the optimum conditions, a calibration graph was obtained over the concentration range 0.005–$3.5\,\mu g\,ml^{-1}$ with a limit of detection (S/N = 2) of $1\,ng\,ml^{-1}$. The correlation coefficient was 0.99993 ($n = 8$) with a relative standard deviation of 0.48% for 10 determinations of $1\,\mu g\,ml^{-1}$ of drug. The method was successfully applied to the determination of metoclopramide in pharmaceutical preparations and biological fluids after IP administration of $25\,mg\,kg^{-1}$ dose to rats. The elimination half-life was $2.5 \pm 0.4\,h$.
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1. Introduction

Metoclopramide, 4-amino-5-chloro-2-methoxy-*N*-(2-diethylamino-ethyl) benzamide, is a dopamine-receptor antagonist, an antiemetic and a stimulant of upper gastrointestinal motility. It is used for the management of gastrointestinal motility disorders and gastrointestinal reflux and for the prevention of cancer chemotherapy-induced emesis at much higher doses [1].

The wide applications of metoclopramide in both clinical and experimental medicine have prompted extensive literature on its determination in dosage forms and in biological fluids. Both the British Pharmacopoeia (BP) [2] and the United States Pharmacopoeia (USP) [3] recommend a nonaqueous acid–base titration with potentiometric detection of the end-point for the evaluation of the raw material of metoclopramide; for its dosage forms, the BP describes

spectrophotometric methods while the USP recommends HPLC methods.

Most of the analytical methods employed for the determination of metoclopramide in dosage forms or in biological fluids are chromatographic methods including HPLC [4–13], LC [14], reversed-phase HPLC [15], HPTLC [16], GC–MS [17], electron-capture GC [18], high-performance capillary electrophoresis (HPCE) [19,20]. Also, spectrophotometric methods are among the most analytical methods used for metoclopramide determination in its dosage forms, in biological fluids or in mixtures with other drugs [21–31]. Other reported methods include titrimetry [32–34], colorimetry [35–37], fluorimetry [38,39], voltammetry [40], ^{1}H-NMR spectroscopy [41], flameless atomic absorption spectrophotometry [42] and radio-immunoassay [43].

Reviewing the literature revealed that up to the present time, nothing has been published concerning the chemiluminescence (CL) determination of metoclopramide hydrochloride.

CL reactions have been widely used for sensitive and selective detection in flow-injection and chromatographic

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analysis [44]. One of the most interesting series of CL reactions is the one involving (2,2′-dipyridyl) ruthenium(II) [Ru(dipy)$_3^{2+}$]. This reaction involves the oxidation of Ru(dipy)$_3^{2+}$ to Ru(dipy)$_3^{3+}$, which is then followed by reduction with an analyte species with the subsequent emission of light [45]. There have been a variety of methods employed to obtain the active oxidized reagent Ru(dipy)$_3^{3+}$. These include chemical/photochemical, electrochemical oxidation and in situ electrochemiluminescence [45]. The chemical generation of Ru(dipy)$_3^{3+}$ has been achieved by a range of reagents, including cerium(IV) sulfate [46], lead dioxide [47] and potassium permanganate [48].

Ru(dipy)$_3^{3+}$ CL methods have been reported for the determination of some drugs, e.g. hydralazine [49], erythromycin [50], clindamycin [51], codeine and heroin [52], 6-mercaptopurine [53], β-lactam antibiotics [54], mefenamic and flufenamic acids [55], some fluoroquinolones [56], and some thioxanthene derivatives [57].

The present paper describes the development of a simple flow-injection (FI) chemiluminometric method for the determination of metoclopramide hydrochloride based on the CL reaction of the drug with Ru(dipy)$_3^{2+}$ and potassium permanganate in a sulfuric acid medium. This method has been satisfactorily applied to the determination of metoclopramide hydrochloride in dosage forms and biological fluids after IP administration of 25 mg kg^{-1} dose in rats.

2. Experimental

2.1. Apparatus and manifold

The flow system used for the determination and CL detection of metoclopramide hydrochloride is shown schematically in Fig. 1. A Gilson Minipuls 3MP4 peristaltic pump (two channels, variable speed) 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 allows mixing of the sample with an acidified 1×10^{-3} M KMnO$_4$ solution, and then combination with 1×10^{-2} M Ru(dipy)$_3^{2+}$ solu-

tion just before the detector. The emitted light was measured by a photomultiplier tube (PMT) (THORN EMI, 9789 QB). The potential supply for PMT was 900 V, provided by a stable power supply (THORN EMI, Model PM 28 BN). The signal was recorded by a Kipp and Zonen BD 112 recorder. Peak height was measured for each signal and expressed as voltage output of the photomultiplier tube. The analytical signal was calculated as sample output minus blank output.

2.2. Reagents and materials

All the reagents used were of analytical reagent grade, and the solutions were prepared with highly distilled water. Aqueous potassium permanganate (Fluka, UK) stock solution of 1×10^{-3} M was prepared in 0.5 M sulfuric acid (Riedel-de Haen). Aqueous Ru(dipy)$_3^{2+}$ (Aldrich Chemical Co.) solution of 1×10^{-2} M was prepared by dissolving Ru(dipy)$_3^{2+}$ hexahydrate in distilled water. Aqueous 0.1 M, 5% sodium hydroxide (Winlab, UK) and 0.1 M hydrochloric acid (Riedel-de Haen) solutions were used. Other reagents used were chloroform (BDH, UK) and dichloromethane (Riedel-de Haen). Metoclopramide hydrochloride was kindly provided by Memphis Chemical Company (Cairo, Egypt). Dosage forms were obtained from local sources. Serum (Multi-Serum-Normal, Randox Laboratories, UK) samples were obtained from rats and commercial sources. Urine samples were obtained from healthy volunteers.

2.3. Standard preparation

Stock solution (1.0 mg ml^{-1}) of metoclopramide hydrochloride was prepared by dissolving 100 mg of the drug sample in 100 ml of distilled water. This solution was further diluted daily with water to give the appropriate concentration for the working solution.

2.4. General procedure

The FI manifold described in Fig. 1 was used. A 250 μl drug solution was injected into a carrier stream of acidified KMnO$_4$ solution, which was then combined with a stream of Ru(dipy)$_3^{2+}$ solution; the resulting peak height was
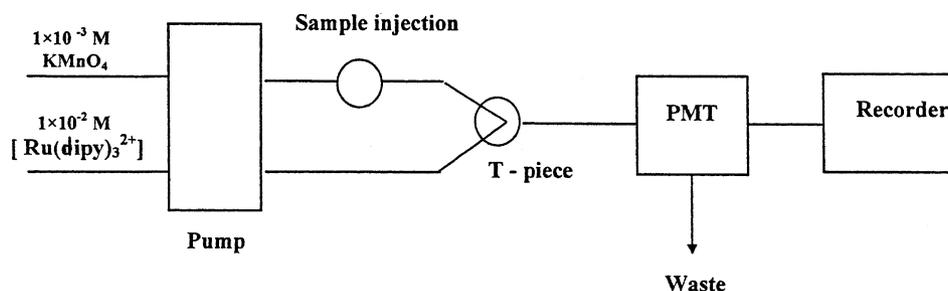

Fig. 1. Flow-injection manifold for CL determination of metoclopramide hydrochloride.

measured. A calibration graph was constructed by plotting the peak height against the drug concentration.

2.5. Procedure for dosage forms

2.5.1. Tablet

Ten tablets were weighed and powdered. Accurately 10 weighed amounts of powder, equivalent to 10 mg each of the drug, were dissolved in distilled water. The solution was filtered and the filtrate was diluted to 100 ml with distilled water. The procedure was followed as described above for calibration. Nominal content of tablets was calculated either from a previously plotted calibration graph or by using the regression equation. Each dilution was measured in triplicates.

2.5.2. Syrup

An accurately measured volume of syrup equivalent to 10 mg of the drug was treated with 20 ml of aqueous 5% NaOH and extracted with chloroform (2×20 ml). The combined extracts were evaporated to dryness at room temperature, the residue was dissolved in 2 ml of 0.1 M HCl and the volume was adjusted to 100 ml with water then preceded as described above. Each dilution was measured in triplicates. The nominal contents of the syrup were calculated either from a previously plotted calibration graph or using the regression equation.

2.6. Procedure for spiked urine and serum

An aliquot of a standard aqueous solution of metoclopramide hydrochloride containing (0.01–$3.5\ \mu g\ ml^{-1}$) was added to 0.5 ml of urine or serum sample in a centrifuge tube and vortex for 20 s. Fifty microliters of 0.1 M NaOH solution was added, shaken for few seconds, followed by the addition of 2.5 ml dichloromethane. The mixture was vortex mixed at high speed for 2 min, and then centrifuged at 3000 rpm for 10 min. The resulting supernatant was transferred to a small conical flask. The extract was evaporated to dryness at $60\ ^\circ$C and the residue was dissolved in 0.5 ml water then analyzed according to the recommended procedure. The absolute recovery was determined by comparing the representative peak height of extracted urine or serum sample with the peak height of the drug at the same concentrations extracted from water.

2.7. Rats and dosing scheme

Three male Sprague–Dawely rats (125–150 g) were used to demonstrate the applicability of the assay to real samples. Metoclopramide was given to the rats as 25 mg kg^{-1} IP doses. Food and water were available ad libitum at all times during the experiment. Rats were lightly anesthetized with Halothane only during blood sampling. Blood samples were collected from the orbital venous plexus 30 min, 1, 2, and 3 h after drug administration. Therefore, each data point

is the mean of three replicates. Serum samples were separated by centrifugation at 6000 rpm for 15 min and stored at $-20\ ^\circ$C till assayed as described above.

3. Results and discussion

As reported earlier [45–48,58], common oxidants such as nitric acid, lead dioxide, cerium(IV), chlorine and potassium permanganate have been used to efficiently produce $Ru(dipy)_3^{3+}$ from $Ru(dipy)_3^{2+}$. In this work, trials were made using different oxidants, such as potassium iodate, potassium bromate, potassium dichromate, potassium perchlorate, potassium persulfate, potassium permanganate, *N*-bromosuccinimide, cerium(IV) sulfate, and hydrogen peroxide, in an acidic or basic medium. The only CL signal was obtained on using $KMnO_4$ and Ce(IV) in acidic medium. The CL intensity obtained with $KMnO_4$ is greater than that obtained with Ce(IV). Thus, $KMnO_4$ is used to produce $Ru(dipy)_3^{3+}$ from $Ru(dipy)_3^{2+}$ which can be used for determination of metoclopramide hydrochloride by the FI technique.

3.1. Configuration designs

A two-line manifold was used for the determination of metoclopramide hydrochloride. The maximum CL intensity was obtained when the sample was injected into a stream of acidified $KMnO_4$, and then mixed with $Ru(dipy)_3^{2+}$ prior to the detector.

3.2. Optimization of experimental variables

The optimum reaction conditions for metoclopramide hydrochloride determination were investigated by injecting 250 μl of 1.0 μg ml^{-1} of the drug solution into the carrier stream with a flow rate of 1.3 ml min^{-1} in each channel. A series of experiments were conducted to establish the optimum analytical variables. The parameters optimized included reagent concentrations and some manifold parameters.

3.2.1. Optimization of reagent concentrations

The effects of varying the concentrations of sulfuric acid as a diluent for potassium permanganate, potassium permanganate and $Ru(dipy)_3^{2+}$ were tested.

The effect of sulfuric acid concentration as a diluent for $KMnO_4$ was studied in the range 1×10^{-5} to 2.0 M. The greatest CL response was obtained with 0.5 M H_2SO_4, above which the intensity was decreased (Fig. 2).

The effect of potassium permanganate concentration on CL intensity of the studied drug is shown in Fig. 3. The greatest CL response was obtained with 1×10^{-3} M $KMnO_4$. Higher concentration of $KMnO_4$ lowered the CL intensity.

The concentration of $Ru(dipy)_3^{2+}$ was studied in the range 1×10^{-5} to 0.1 M. Peak height increased with increasing $Ru(dipy)_3^{2+}$ concentration. Fig. 4 shows that 1×10^{-2} M
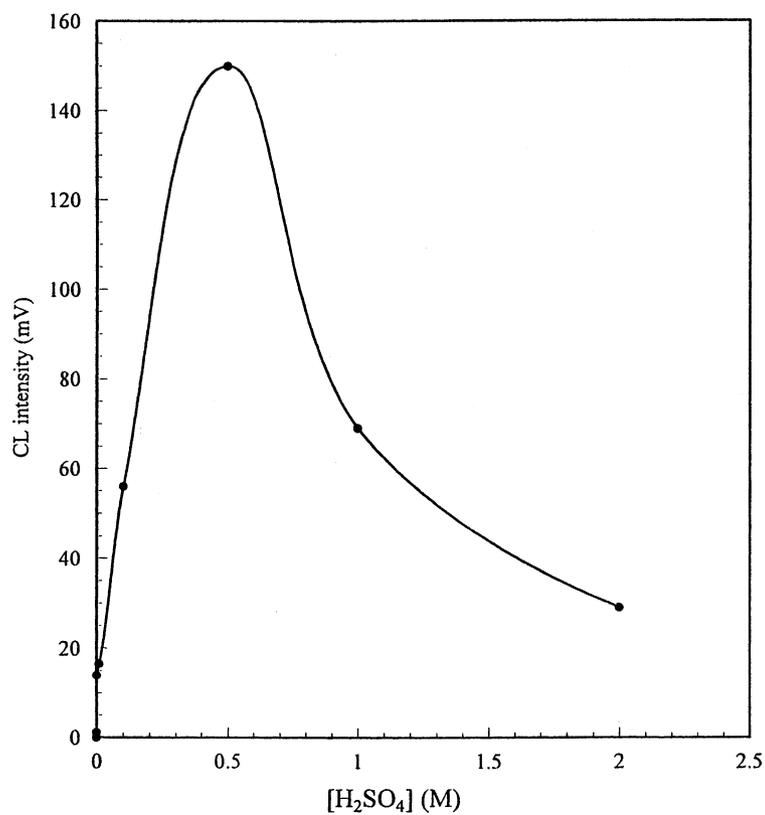
Fig. 2. Effect of sulfuric acid concentration as a diluent for $KMnO_4$ on the CL intensity of metoclopramide hydrochloride (1 μg ml^{-1}), $KMnO_4$ 1×10^{-3} M, Ru(dipy)$_3$$^{2+}$ 1×10^{-2} M, total flow rate 2.6 ml min^{-1}.

Fig. 3. Effect of $KMnO_4$ concentration on CL intensity of metoclopramide hydrochloride (1 μg ml^{-1}), Ru(dipy)$_3$$^{2+}$ 1×10^{-2} M, total flow rate 2.6 ml min^{-1}.
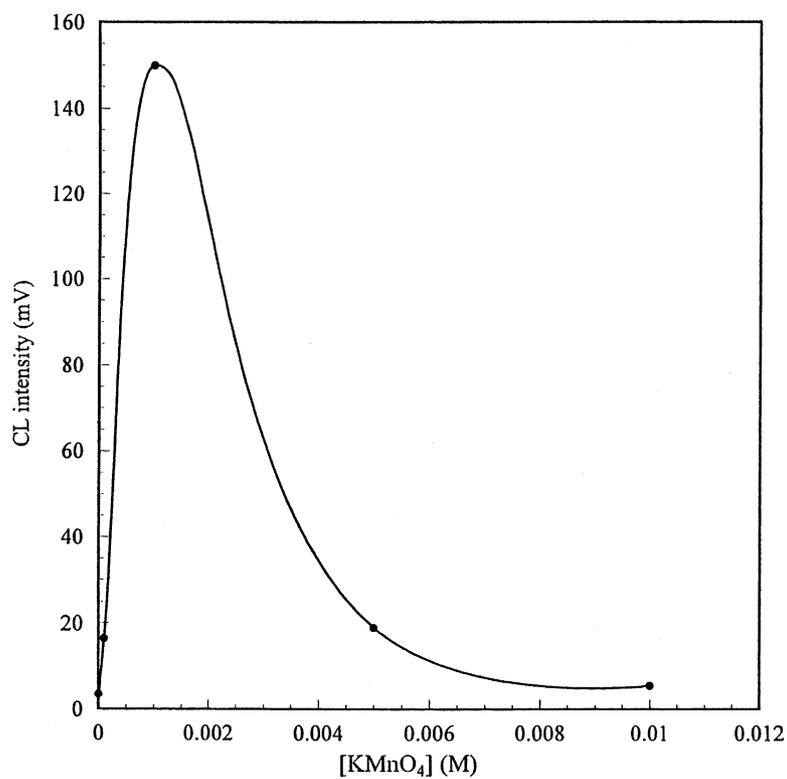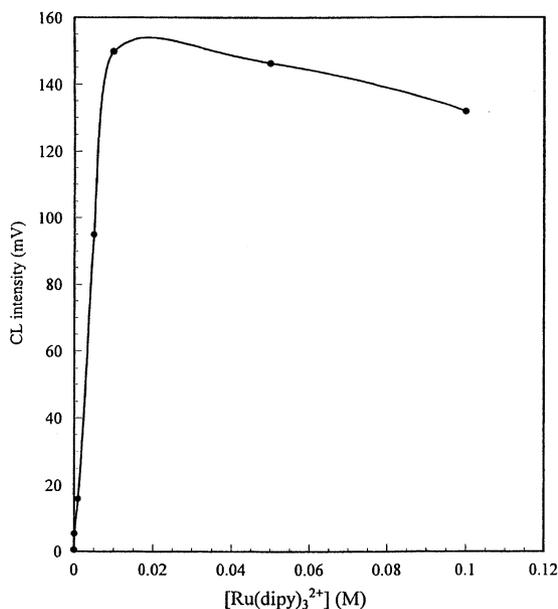
Fig. 4. Effect of $Ru(dipy)_3^{2+}$ concentration on the CL intensity of metoclopramide hydrochloride ($1 \mu g \, ml^{-1}$), $KMnO_4$ 1×10^{-3} M, total flow rate $2.6 \, ml \, min^{-1}$.

gave the greatest intensity, above which the intensity was slightly decreased.

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

3.2.2. Optimization of manifold parameters

The manifold parameters studied under the optimized reagent concentrations were the injected sample volume and the flow rate.

The volume injected was varied between 30 and 600 μl. The resulting peak height increased with increasing volumes injected up to 250 μl, above which the intensity was slightly decreased (Fig. 5).

The total flow rates of the $Ru(dipy)_3^{2+}$ and potassium permanganate streams were varied over the range $0.7–7.0 \, ml \, min^{-1}$, with equal flows in each channel. The results obtained show that $2.6 \, ml \, min^{-1}$ ($1.3 \, ml \, min^{-1}$ for each channel) was the best total flow rate for the studied drug, above which the intensity decreased continuously (Fig. 6).

3.3. Possible CL mechanism

$Ru(dipy)_3^{2+}$ CL has proven to be a very sensitive detection system for compounds which contains a secondary or tertiary aliphatic amine [59]. The studied drug contains amino and diethyl amino groups, thus the proposed reaction mechanism is presumably similar to that reported previously for amine determination utilizing its electrogenerated CL reaction with $Ru(dipy)_3^{2+}$ [59,60]. The proposed mechanism
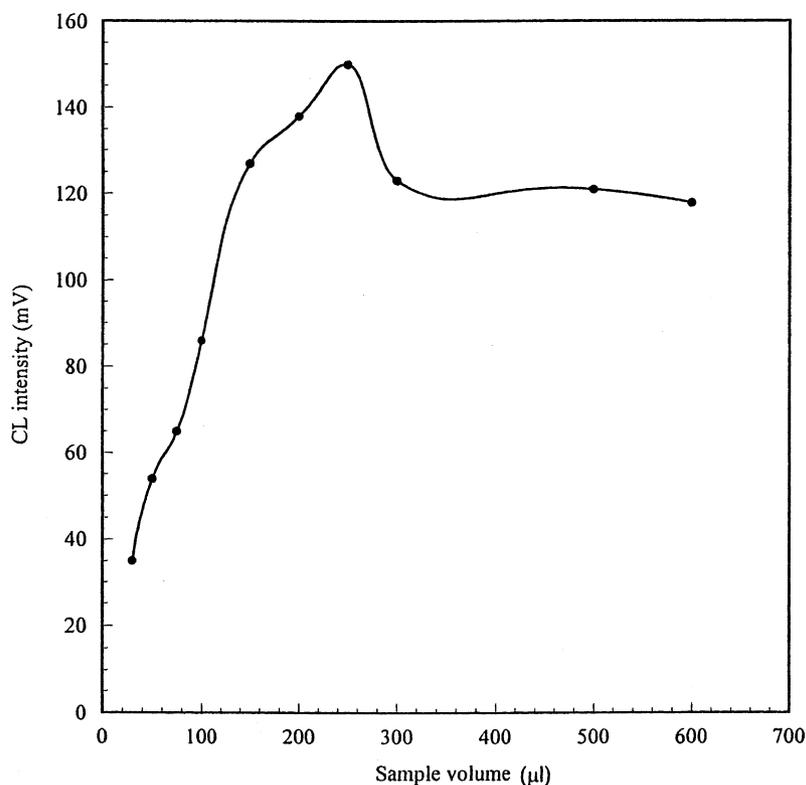

Fig. 5. Effect of sample volume on the CL intensity of metoclopramide hydrochloride ($1 \mu g \, ml^{-1}$), $KMnO_4$ 1×10^{-3} M, $Ru(dipy)_3^{2+}$ 1×10^{-2} M, total flow rate $2.6 \, ml \, min^{-1}$.
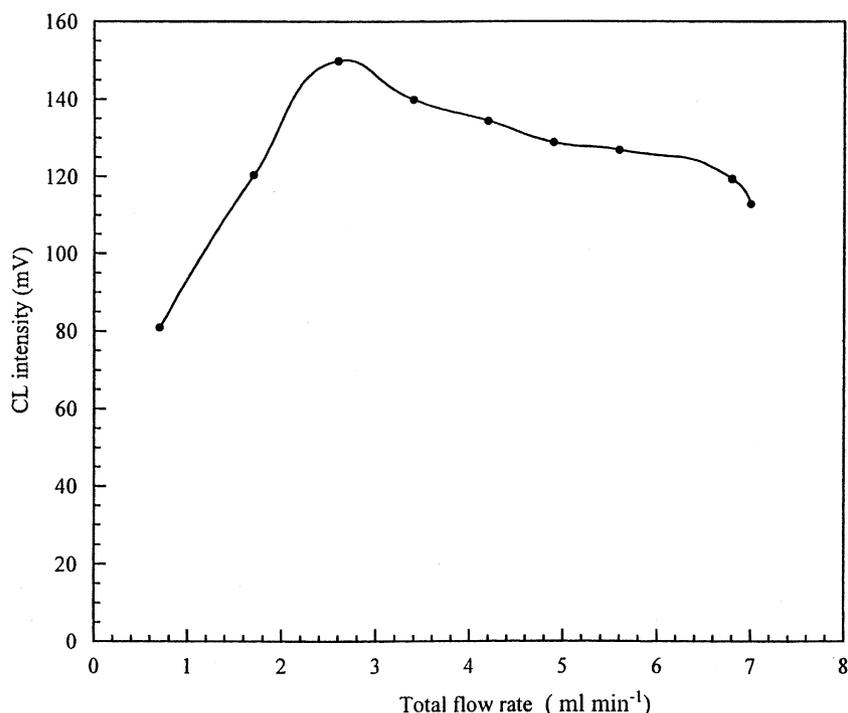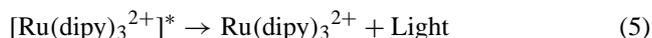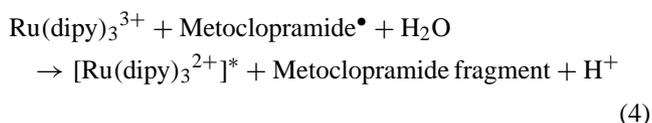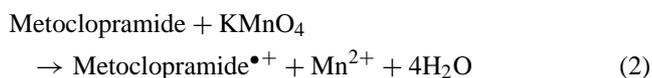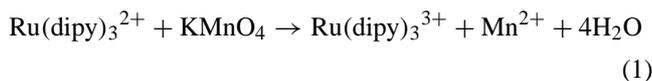
Fig. 6. Effect of total flow rate on the CL intensity of metoclopramide hydrochloride (1 μg ml^{-1}), KMnO$_4$ 1 × 10^{-3} M, Ru(dipy)$_3^{2+}$ 1 × 10^{-2} M.

involves the oxidation of Ru(dipy)$_3^{2+}$ and the primary amine present on metoclopramide by potassium permanganate since the resultant radical ion is more stable by resonance. The oxidation product of amine undergoes deprotonation to form a radical. This reduces the Ru(dipy)$_3^{3+}$ to the excited state that subsequently emits light, shown as follows:

$$Ru(dipy)_3^{2+} + KMnO_4 \rightarrow Ru(dipy)_3^{3+} + Mn^{2+} + 4H_2O \tag{1}$$

$$\begin{aligned} Metoclopramide &+ KMnO_4 \\ &\rightarrow Metoclopramide^{\bullet+} + Mn^{2+} + 4H_2O \end{aligned} \tag{2}$$

$$Metoclopramide^{\bullet+} \rightarrow Metoclopramide^{\bullet} + H^+ \tag{3}$$

$$\begin{aligned} Ru(dipy)_3^{3+} &+ Metoclopramide^{\bullet} + H_2O \\ &\rightarrow [Ru(dipy)_3^{2+}]^* + Metoclopramide\ fragment + H^+ \end{aligned} \tag{4}$$

$$[Ru(dipy)_3^{2+}]^* \rightarrow Ru(dipy)_3^{2+} + Light \tag{5}$$

3.4. Determination of metoclopramide hydrochloride

After chemical and instrumental variables were optimized for maximum CL intensity, a series of standard solutions was pumped, each as three replicates, to test the linearity of the calibration graph. The CL intensity (I, mV) was linearly related to metoclopramide hydrochloride concentration over the range of 0.005–3.5 μg ml^{-1} with a minimum detectability (S/N = 2) of 0.001 μg ml^{-1} as to be valuable for detect-

ing the studied drug in biological fluids. Linear regression analysis of the results gave the following equation:

$$I = 2.192 + 95.98C \quad (r = 0.99993, n = 8).$$

The standard deviations of slope and intercept were 0.48 and 0.75, respectively.

The precision of the method was evaluated by analyzing standard solutions of metoclopramide. The average % recovery was 99.9 ± 1.7 (mean ± S.E.).

The reproducibility was investigated using 1.0 μg ml^{-1} for the drug ($n = 10$) and the percent of relative standard deviation (%R.S.D.) value was 0.48% which illustrates that the results were highly reproducible.

3.5. Analysis of pharmaceutical preparations

The proposed method was further applied to the analysis of certain dosage forms containing metoclopramide hydrochloride in order to evaluate the analytical usefulness of the chemiluminescent method. Very good results with excellent recoveries were obtained based on three determinations for different concentrations of each pharmaceutical preparation. The results in Table 1 are in accordance with those obtained by the fluorimetric method [39]. The results indicate that methyl and propylparaben did not interfere during the determination of metoclopramide hydrochloride in premperan syrup. Statistical analysis [61] of the results by using t-test and F-test showed no significant difference between the two methods as regards to accuracy and precision.

Table 1
Analysis of metoclopramide hydrochloride in pharmaceutical preparations by the proposed and the fluorimetric methods

Preparation	Concentration taken ($\mu g\,ml^{-1}$)	Recovery (%)	
		Proposed method	Flourimetric method [39]
Premperan tablets[a] (10 mg metoclopramide hydrochloride/tablet)	0.01	100.0	
	0.05	99.7	
	0.1	101.7	
	1.0	100.1	
	2.0	99.9	
	3.0	100.0	
Mean ± S.D.		100.2 ± 0.7	100.4 ± 0.6
Plasil tablets[b] (10 mg metoclopramide hydrochloride/tablet)	0.01	99.7	
	0.05	101.4	
	0.1	100.0	
	1.0	100.0	
	2.0	100.1	
	3.0	100.0	
Mean ± S.D.		100.2 ± 0.6	100.3 ± 0.7
Premperan syrup[a] (100 mg metoclopramide hydrochloride + 150 mg methyl and propylparaben)	0.01	100.0	
	0.05	100.0	
	0.1	100.8	
	1.0	99.9	
	2.0	100.0	
	3.0	100.1	
Mean ± S.D.		100.1 ± 0.3	101 ± 1

[a] Product of Synthelabo Laboratories, France.
[b] Product of Lepetit, Milan, Italy.

3.6. Analysis of spiked urine and serum samples

The high sensitivity attained by the proposed method allows the determination of metoclopramide hydrochloride in biological fluids. Metoclopramide is rapidly absorbed from the gastro-intestinal tract and has been reported to undergo a high degree of first-pass hepatic metabolism. It is excreted in the urine as free and as conjugated metoclopramide and as metabolites [62]. Following administration of metoclopramide 10 mg by mouth, about 78% of the dose was excreted in the urine in the first 24 h. Blood concentrations of metoclopramide reached peak concentration of
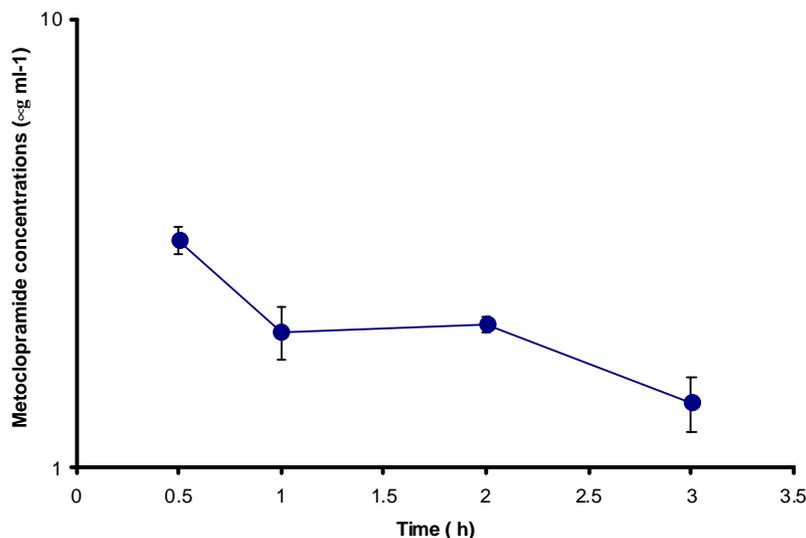

Fig. 7. Serum–drug concentration time profile (mean ± S.E.) after IP administration of metoclopramide (25 mg kg^{-1}) to three rats.

Table 2
Determination of metoclopramide hydrochloride in spiked urine and serum samples

Concentration taken (μg ml^{-1})	Found (%)	
	Urine	Serum
0.01	100.0	100.0
0.05	100.0	99.3
0.1	99.6	99.6
0.5	99.4	99.4
1.0	100.0	99.4
2.0	100.8	100.6
3.0	100.0	100.8
Mean $\pm$ S.D.	100.0 $\pm$ 0.4	99.9 $\pm$ 0.6

about 40 ng ml^{-1} within the first 2 h and the half-life of the drug for the period between 3 and 8 h following the dosage was 4 h [62].

Extraction was required to avoid any interference. The extraction process was carried out with dichloromethane after alkalinization with an aqueous solution of sodium hydroxide. The extraction procedure for biological fluids was performed by using some different solvents, dichloromethane, was found to be the best extraction solvent for metoclopramide hydrochloride in biological fluids [4] which gave excellent recoveries for urine and serum samples with the proposed procedure. (Table 2) depicts a Fig. 7 typical plot of metoclopramide concentrations after IP administrations (25 mg kg^{-1}) to three rats. The determined concentrations were comparable to a previously published work [4] which demonstrates the usefulness of this method for analysis of metoclopramide in serum.

4. Conclusion

A simple, rapid and highly sensitive chemiluminometric method is described for the determination of metoclopramide hydrochloride in dosage forms and in biological fluids. Solutions can be analyzed at a rate of 67 samples h^{-1}. The proposed method is characterized by its simplicity compared with the official methods. The USP [3] recommends a HPLC method for dosage forms that requires special skill to obtain reliable results, consume more organic solvents and have longer run time. The BP [2] recommends a spectrophotometric method for metoclopramide determination in its dosage forms in nonaqueous media. The method described herein has sufficient sensitivity to determine the pharmacokinetics of metoclopramide following a single IP dose.

Acknowledgements

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