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Development and Validation of an Ecofriendly Chemiluminescence Method for the Determination of Citalopram in Pharmaceutical Preparations using Cu^{2+}-grafted Oxidized Multiwall Carbon Nanotubes

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ABSTRACT

A novel and sensitive chemiluminescence (CL) method was developed and validated for the determination of citalopram in bulk dosage form and in pharmaceutical preparations. The method is based on the use of Cu^{2+}-grafted oxidized multiwall carbon nanotubes (Cu/ox-MWCNTs). The weak CL signal arising from the reaction of an alkaline luminol-H_2O_2 system was significantly enhanced by the addition of citalopram in the presence of ox-MWCNTs/Cu^{2+}. Furthermore, ox-MWCNTs/Cu^{2+} exhibited exceptional catalytic activity towards the oxidation of luminol in the luminol-H_2O_2 CL reaction. Multiwalled carbon nanotubes (MWCNTs) were characterized by scanning electron microscopy (SEM), which also showed the attachment of Cu^{2+} to MWCNTs. Various factors affecting CL intensity were carefully investigated and optimized for citalopram quantitation. The CL intensity was proportional to citalopram concentration in the range 0.2–8.0 μg mL^{-1}, with a correlation coefficient of 0.996. The limit of detection (LOD) and limit of quantification were 2.29×10^{-5} μg mL^{-1} and 7.64×10^{-5} μg mL^{-1}, respectively, and its reproducibility was satisfactory with a relative standard deviation (RSD) of 2.59 % (n = 5). The interference effects of common excipients were studied, and the developed method was effectively applied for the determination of citalopram in pure form and in pharmaceutical preparations. Percentage recoveries were calculated and ranged from 98.67 to 101.46 % for the pure form and from 97.38 to 101.72 % for pharmaceutical preparations.

KEYWORDS

Chemiluminescence, Citalopram, Luminol, oxidized multiwall carbon nanotubes.

1. Introduction

Citalopram (CIT; 1- (3-dimethylaminopropyl)-1-(4-fluorophenyl)-1, 3-dihydroisobezofuran-5-carbonitrile)[1] is a second generation selective serotonin reuptake inhibitor, which belongs to the group of drugs known as antidepressants. CIT has broad-spectrum therapeutic activity against depression, anxiety, and obsessive and impulse control disorders.[2,3] It has also been reported to reduce the symptoms of diabetic neuropathy and premature ejaculation [4,5], and may be effective for the treatment of post-stroke pathological crying.[6]

CIT is normally taken as a single daily dose in the morning or evening with or without food. Food does not increase the absorption of CIT but helps to prevent nausea. In the therapeutic dose range, CIT is safe and well tolerated. Recommended doses range from 20 to 40 mg daily, which results in a plasma concentration range from 30–130 ng mL^{-1}.[7] Overdosage may cause drowsiness, vomiting, dizziness, sweating, nausea, coma, confusion, and heartbeat anomalies.[8]

Citalopram is absorbed by cytochrome P to its demethylated metabolites, desmethylcitalopram and didesmethylcitalopram. Desmethylcitalopram is the chief metabolite and can also prevent 5-HT reuptake, but is less potent than citalopram.[9] Until recently, citalopram was sold as a racemic mixture, consisting of 50 % *R*-(–)-citalopram and 50 % *S*-(+)-citalopram, but as the *S*-(+)-enantiomer has the desired antidepressive effect[10], it was recently introduced under the generic name escitalopram.

Various analytical techniques have been developed to determine citalopram levels in commercial formulations and biological samples. These include gas chromatographic, gas chromatographic/mass spectrometric (GC/MS)[11,12], high performance liquid chromatographic (HPLC) with fluorescence[13–16], UV[16–18], and MS detectors [19,20], and electrophoretic methods.[21–26] Spectrofluorimetric[27–30] and spectrophotometric[31–33] methods have also been reported for the citalopram determination in pharmaceutical formulations. Few CL methods have been recently reported for the determination of citalopram in pharmaceutical preparations and biological samples.[34,35]

Chemiluminescence (CL) is a powerful analytical technique with widespread applications in numerous fields because of its high sensitivity, rapidity, wide linear range, high signal to noise ratios (due to the absence of a light source), instrumental simplicity and affordability, and the comparative absence of toxic effects.[36–41]

On the other hand, carbon nanotube (CNT) materials are attracting considerable research interest. In particular, in addi-

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tion to being small sizes, CNTs have huge surface areas, hollow or layered structures, high mechanical strengths, and significant electrical conductivities. These properties are of interest in various applications, such as, field emission, reinforcing materials for composites, nanoprobes, and chemical sensors.[42–46] CNTs are available as singled-walled carbon nanotubes or multiwall carbon nanotubes (MWCNTs) according to the number of layers carbon atom in their walls. Furthermore, when MWCNTs are treated with a mixture of H_2SO_4 and HNO_3, carboxyl groups are produced on their walls and some physical properties are changed. In addition, the presence of oxygen containing functional groups on side walls provides a means of introducing positively charged species.[47–52]

In the present study, a CL-based method involving the use of oxidized multiwall carbon nanotubes (ox-MWCNTs) grafted with Cu^{2+} was devised to quantify citalopram levels in pharmaceutical preparations. We found that ox-MWCNTs/Cu^{2+} enhanced the CL signal arising from the reaction between alkaline luminol and H_2O_2, and that CL intensities were further boosted by the presence of citalopram. Based on these observations, we developed a sensitive CL method for the quantification of citalopram in pharmaceutical preparations. Citalopram was found to markedly increase the CL intensity of the luminol-H_2O_2-ox-MWCNTs/Cu^{2+} system and this enhance was proportional to the concentration of citalopram present. After optimizing experimental parameters, CL intensity was found to be linear to citalopram concentration in the range 0.2–8.0 μg mL^{-1} with a detection limit of 2.29×10^{-5} μg mL^{-1}.

2. Experimental

2.1. Materials and Reagents

Reagents used were of high grade purity and double-deionized water (DI) was used throughout. Luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione) was purchased from Sigma-Aldrich (St. Louis, Missouri, US). Hydrogen peroxide and sodium hydroxide were from the Junsei Chemical Co., Ltd. Quality Assurance Section 1–6, Ohmano-cho, Koshigaya-shi, Saitama, 4-0844, Japan. MWCNTs (>93 % purity, 10–40 nm average diameter, and 1–25 μm long) were brought from CNT (Daegu, South Korea). Standard reference citalopram was provided by Z-Jan's Pharmaceutical Industry (Peshawar, Pakistan). Commercial formulations of citalopram, Lopram 20 mg tablets (manufactured by Hansel Pharmaceuticals (Pvt) Ltd, Lahore, Pakistan), Citalo 20 mg tablets (manufactured by Platinum Pharmaceuticals (Pvt) Ltd, Karachi, Pakistan) and Pramcit 20 mg tablets (manufactured by Nabiqasim Industries (Pvt) Ltd, Karachi, Pakistan) were purchased locally.

2.2. Preparation of Reagents and Sample Solutions

Luminol stock solution (4×10^{-3} M) was prepared by dissolving 0.035 g of luminol in 4.5 mL of 0.1 M NaOH, diluted to 50 mL with deionized (DI) water, and stored at 4 °C. Hydrogen peroxide solution (4 M) was prepared by diluting 10.213 mL of 30 % H_2O_2 to 25 mL with DI water. Sodium hydroxide (0.1 M) solution was prepared by dissolving 2.0 g of NaOH in 50 mL DI water. Working solutions were prepared freshly before use from stock solutions by proper dilution with DI water. A standard stock solution of citalopram (250 μg mL^{-1}) was prepared by dissolving 0.0125 g of citalopram standard in 2 mL of distilled ethanol, and diluting to 50 mL with DI water. Working solutions of 10 μg mL^{-1} and 5 μg mL^{-1} citalopram were prepared daily by diluting proper amounts of the stock solution with DI water.

2.3. Synthesis of Oxidized MWCNTs

Ox-MWCNTs were prepared as previously described by Rosca *et al*.[53,54] Briefly, 0.5 g pristine MWCNTs (p-MWCNTs) were added to 200 mL nitric acid solution in a round-bottomed glass flask. p-MWCNTs were dispersed by sonication for 1 h using an ultrasonic bath (JAC Ultra-sonic 4020p, KODO, South Korea), and the mixture was then refluxed at 120 °C for 12 h under nitrogen. After cooling, the MWCNTs were washed and filtered several times using DI water and a 0.22 μm membrane nitrocellulose filter (Millipore, USA). The MWCNTs so obtained were then dried in an oven at 60 °C for 48 h (Fig. 1).

2.4. Synthesis of Cu^{2+}-grafted Oxidized Multiwall Carbon Nanotubes

Oxidized MWCNTs (0.1 g) were added to 20 mL of a 1×10^{-3} M Cu (II) solution and dispersed by sonication for 30 min. After cooling, the ox-MWCNTs/Cu^{2+} were washed and filtered through a nitrocellulose filter (0.20 μm; Millipore, USA). The obtained ox-MWCNTs/Cu^{2+} were then dried at 60 °C for 24 h (Fig. 1).

2.5. Instrument

A Hitachi F-4500 spectrofluorometer was used to detect and record CL produced by the luminol-H_2O_2-ox-MWCNTs/Cu^{2+}

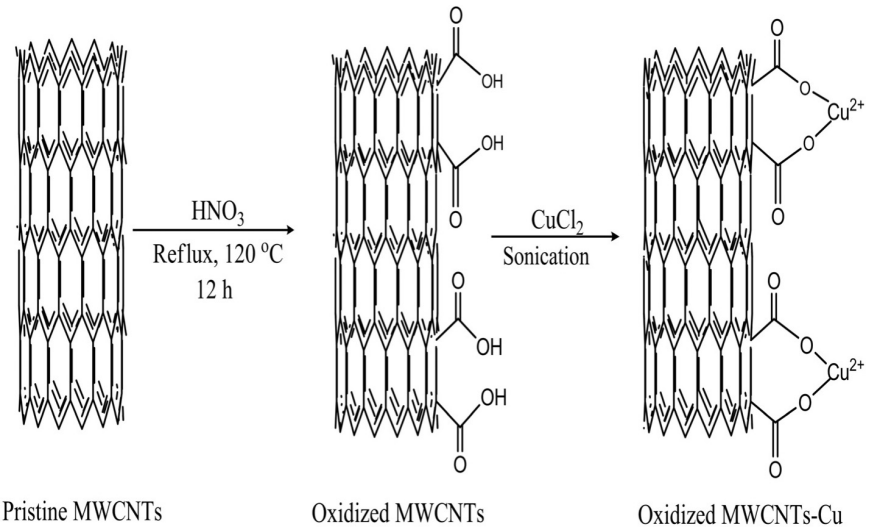

Figure 1 Schematic diagram of the preparation of oxidized MWCNTs and Cu^{2+}-grafted MWCNTs.

system. For CL measurements, the light source of the spectrofluorometer was switched off and only the photomultiplier tube (Model, R 928, Hamamatsu, Japan) was used. The slit width of the emission monochromators was 5 nm and the power supply of the photomultiplier tube used to detect CL signals was 700 V. Light-producing reactions were carried out in a standard 1 × 1 cm quartz cell placed in the spectrofluorometer cell holder.

2.6. Analytical Procedure

Analytical procedure consisted of adding the following quantities of equilibrated solutions to the CL reaction cell using a micropipette. Citalopram stock solution to give final concentration of 0.2–8.0 μg mL^{-1}, 4 × 10^{-4} g ox-MWCNTs/Cu^{2+}, 400 μL of NaOH solution (8 × 10^{-3} mol L^{-1}), and 400 μL luminol (4 × 10^{-3} mol L^{-1}). The kinetic-curve of the CL signal was measured at 430 nm after injecting of 400 μL (4 × 10^{-2} mol L^{-1}) of H_2O_2 into cells to initiate the CL reaction. CL intensities (ΔI) were calculated using $\Delta I = I_s - I_o$, where I_s and I_o are CL signals in the presence and absence of citalopram, respectively.

2.7. Application to Pharmaceutical Preparations

Five tablets each containing 20 mg of active citalopram were weighed and weights were averaged. The tablets were then powdered in a mortar. The powder equivalent of 0.0125 g citalopram was dissolved in 2 mL of ethanol, approximately 10 mL of DI water was added, and mixture sonicated for 10 min. The resultant solution was then filtered and diluted to 50 mL with DI water. An appropriate volume of this solution was then diluted with DI water to provide a citalopram concentration in the working range. A certain volume of this solution was then analyzed using the above described procedure and amount of citalopram present per tablet was calculated by the calibration equation.

3. Results and Discussion

3.1. Characterization of MWCNTs, Oxidized MWCNTs and Cu^{2+}/ox-MWCNTs

The morphologies of pristine-MWCNTs, ox-MWCNTs, and ox-MWCNTs/Cu^{2+} were examined by scanning electron microscopy (SEM) (S-4200, HITACHI, Japan). The Pristine-MWCNTs appeared as separate thread-like carbon fibres (Fig. 2(a)), whereas ox-MWCNTs were aggregated (Fig. 2(b)), and Cu^{2+} coated ox-MWCNTs were more aggregated (Fig. 2(c)).

3.2. CL Characterization

A CL emission spectrum of the luminol-H_2O_2-citalopram-MWCNTs/Cu^{2+} system is presented in Fig. 3. As shown in Fig. 3(a), the luminol-H_2O_2 system produced a weak CL signal, which was not enhanced by the addition of ox-MWCNTs (Fig. 3(b)). However, the addition of ox-MWCNTs/Cu^{2+} to the luminol-H_2O_2 system augmented the CL signal (Fig 3(c)), and the addition of citalopram markedly enhanced the signals obtained (Fig 3(d)), which exhibited maximum intensity at 430 nm. Furthermore, the CL intensity of the luminol-H_2O_2-citalopram-MWCNTs/Cu^{2+} system was proportional to the concentration of citalopram present.

3.3. Optimization of the Reagents Concentration

The effect of the amount of MWCNTs/Cu^{2+} used was investigated in the range 2–10 × 10^{-4} g. Maximum CL intensity of the luminol-H_2O_2-citalopram-MWCNTs/Cu^{2+} system was achieved at 4 × 10^{-4} g (Fig. 4), and thus, this amount was used in further experiments. The effect of sodium hydroxide concentration on the luminol-H_2O_2-citalopram-MWCNTs/Cu^{2+} system was investigated in the range 2–12 × 10^{-3} mol L^{-1}. CL intensity was found to peak at a NaOH concentration of 8 × 10^{-3} mol L^{-1} (Fig. 5). The

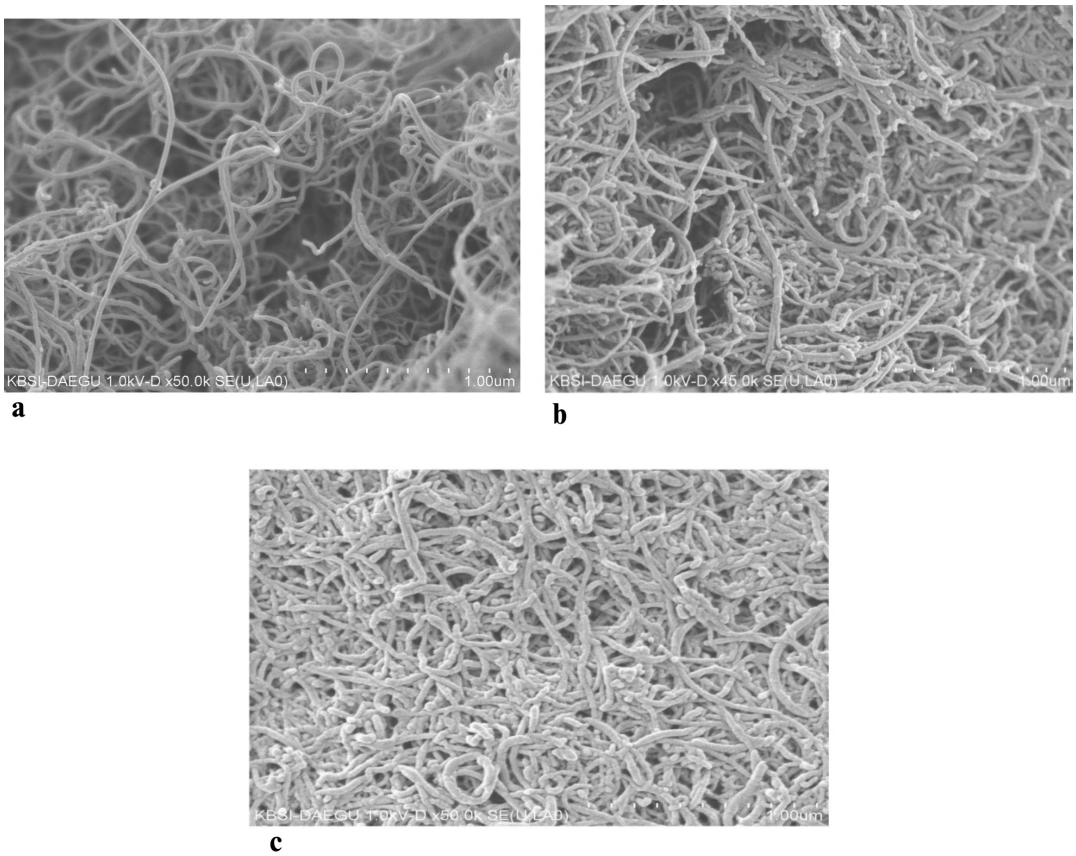

Figure 2 SEM images of (a) Pristine MWCNTs; (b) Oxidized MWCNTs; (c) Oxidized MWCNTs/Cu^{2+}.

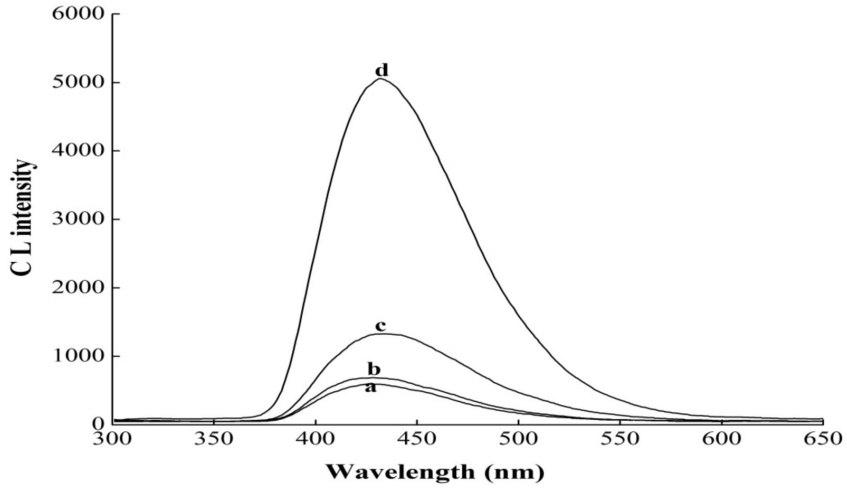

Figure 3 CL emission spectra of; (a) luminol-H_2O_2; (b) luminol-H_2O_2-MWCNTs; (c) luminol-H_2O_2-Cu^{2+}/MWCNTs; (d) luminol-H_2O_2-Cu^{2+}/MWCNTs-citalopram.
Conditions: citalopram, 8.0 μg mL^{-1}; Cu^{2+}/MWCNTs, 4×10^{-4} g; NaOH, 8×10^{-3} mol L^{-1}; luminol, 4×10^{-3} mol L^{-1}; H_2O_2, 4×10^{-2} mol L^{-1}

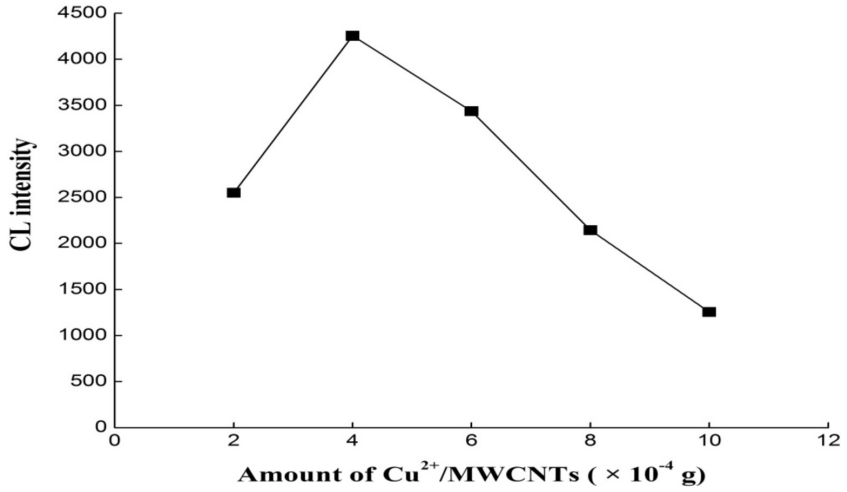

Figure 4 Effect of Cu^{2+}/MWCNTs dosage on CL intensity.
Conditions: citalopram, 8.0 μg mL^{-1}; Cu^{2+}/MWCNTs, 2–10 $\times 10^{-4}$ g; NaOH, 6×10^{-3} mol L^{-1}; luminol, 2×10^{-3} mol L^{-1}; H_2O_2, 3×10^{-2} mol L^{-1}; λ_{eme} 430 nm.

effect of luminol concentration on CL intensity was also studied in the range 1–6 $\times 10^{-3}$ mol L^{-1}. CL intensity peaked at a luminol concentration of 4×10^{-3} mol L^{-1} (Fig. 6). The effects of H_2O_2 concentration were examined in the range 1–6 $\times 10^{-2}$ mol L^{-1}. CL intensity increased with H_2O_2 concentration to 4×10^{-2} mol L^{-1} and then plateaued (Fig 7).

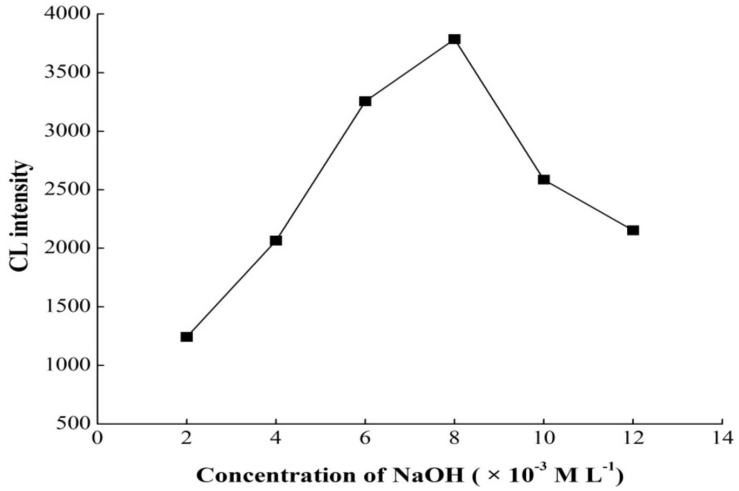

Figure 5 Effect of NaOH concentration on CL intensity.
Conditions: citalopram, 8.0 μg mL^{-1}; Cu^{2+}/MWCNTs , 4×10^{-4} g; NaOH, 2–12 $\times 10^{-3}$ mol L^{-1}; luminol, 2×10^{-3} mol L^{-1}; H_2O_2, 3×10^{-2} mol L^{-1}; λ_{eme} 430 nm.

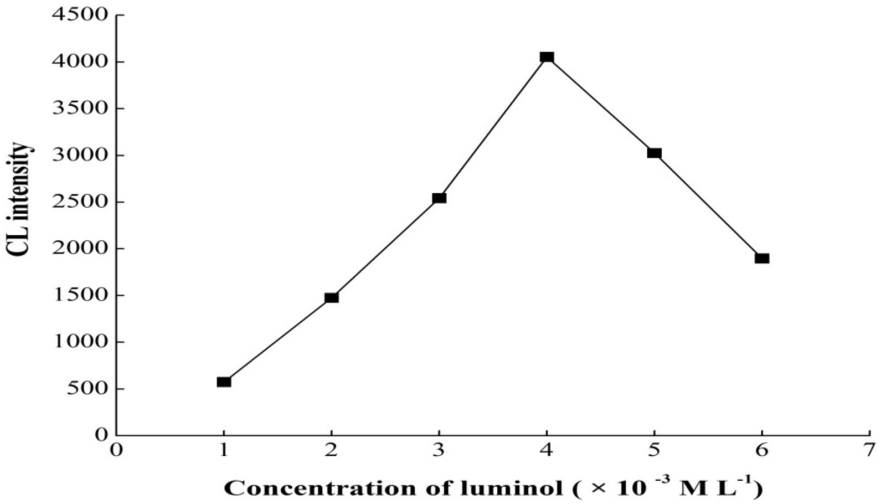

Figure 6 Effect of luminol concentration on CL intensity.
Conditions: citalopram, 8.0 μg mL^{-1}; Cu^{2+}/MWCNTs, 4×10^{-4} g; NaOH, 8×10^{-3} mol L^{-1}; luminol, 1–6 $\times 10^{-3}$ mol L^{-1}; H_2O_2, 3×10^{-2} mol L^{-1}; λ_{eme} 430 nm.

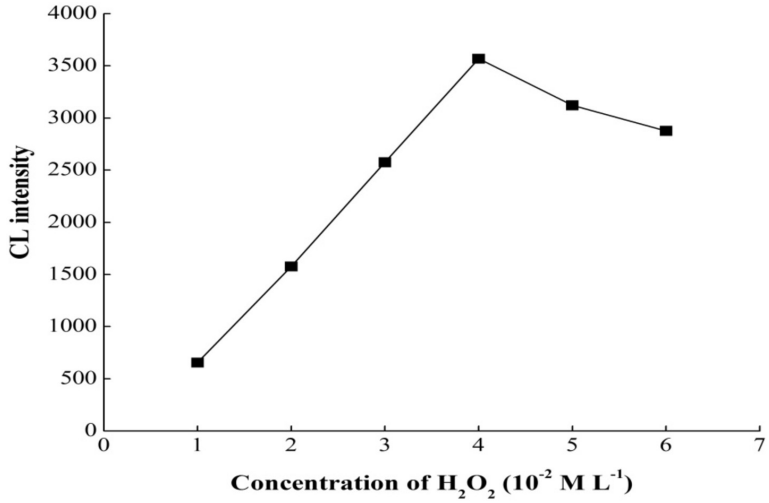

Figure 7 Effect of H_2O_2 concentration on CL intensity.
Conditions: citalopram, 8.0 μg mL^{-1}; Cu^{2+}/MWCNTs, 4×10^{-4} g; NaOH, 8×10^{-3} mol L^{-1}; luminol, 4×10^{-3} mol L^{-1}; H_2O_2, 1–6 $\times 10^{-2}$ mol L^{-1}; λ_{eme} 430 nm.

3.4. Analytical Features of Merit

Under optimum experimental conditions, the concentration of citalopram and chemiluminescence intensity exhibited a linear relationship in the range 0.2–8.0 μg mL^{-1} with a correlation coefficient of 0.996–0.9977 (Fig. 8a,b). Limit of detection (LOD) was defined as the concentration of citalopram that produced a CL intensity three times the blank standard deviation (LOD = 3 S_b/m), whereas limit of quantification (LOQ) was defined as the concentration of the citalopram that produced a CL intensity ten times the blank standard deviation (LOQ = 10 S_b/m). Calculated LOD and LOQ values were 2.29×10^{-5} μg mL^{-1} and 7.64×10^{-5} μg mL^{-1} respectively. The linear regression equation, its slope, intercept, and correlation coefficient, and the relative standard deviation of the response factor are provided in Table 1. The sensitivity of the developed method is compared with other described citalopram methods in Table 2. The table shows that the sensitivity of the devised method is greater than that of any of the previously described methods.

3.5. Interference Effects

Interference by common excipients like glucose, starch, fructose, lactose, magnesium stearate, and talc were studied. The interference study was conducted by preparing samples containing fixed amounts of citalopram (0.2 μg mL^{-1}) and different concentrations (5, 10, 20, and 40-fold concentrations) of excipients. No interferences were observed by these common excipients (Fig. 9).

3.6. Reliability of the Method

The precision of the proposed method was studied by analyzing pure citalopram and pharmaceutical formulations using three different concentrations of each in triplicate. Results are given in Table 3 for pure citalopram and in Table 4 for pharma-

Table 1 Analytical parameters for the chemiluminescence determination of citalopram.

Parameter	Value
λ_{eme} /nm	430
Linear range /μg mL^{-1}	0.2–8.0
Limit of detection /μg mL^{-1}	2.29×10^{-5}
Limit of quantification /μg mL^{-1}	7.64×10^{-5}
Regression equation (y)	Y = 658.5X – 23.7
Slope (b)	658.5
Intercept (a)	23.7
Correlation coefficient /r	0.9977
Standard deviation /μg mL^{-1}	5.02×10^{-3}
Relative standard deviation /%	2.59

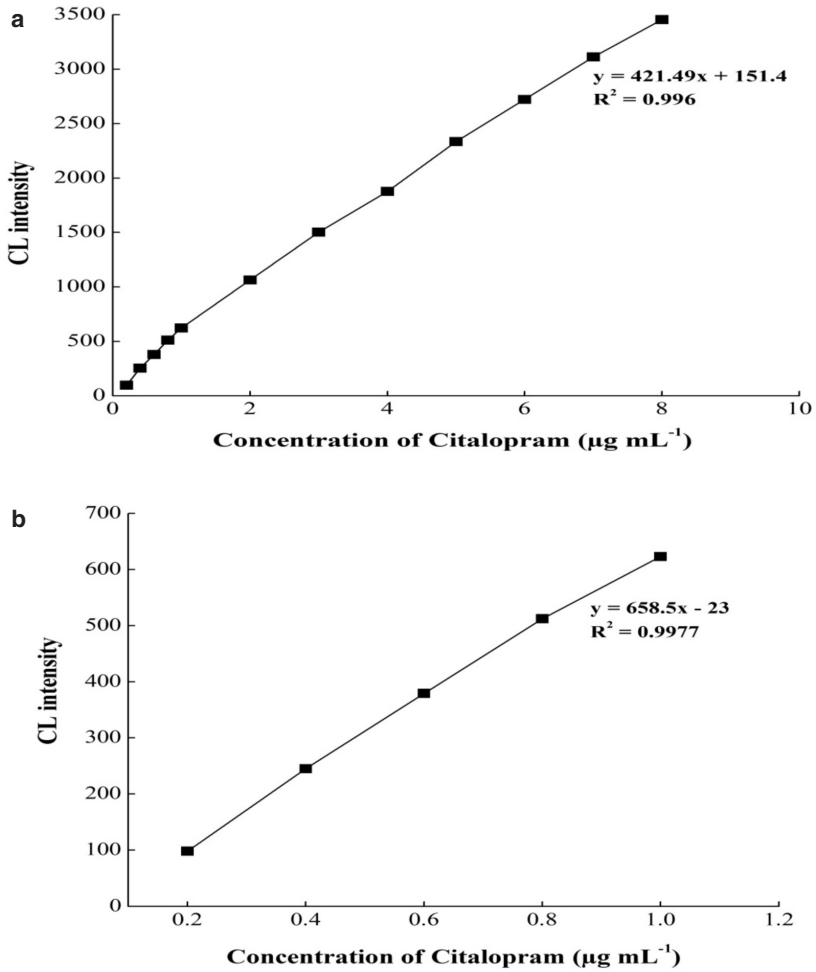

Figure 8 **a,** Linear range of citalopram. **b**, Calibration curve of citalopram.
Conditions for (**a**): citalopram, 0.2–8.0 μg mL^{-1}; Cu^{2+}/MWCNTs, 4×10^{-4} g; NaOH, 8×10^{-3} mol L^{-1}; luminol, 4×10^{-3} mol L^{-1}; H_2O_2, 4×10^{-2} mol L^{-1}; λ_{eme} 430 nm.
Conditions for (**b**): citalopram, 0.2–1.0 μg mL^{-1}; Cu^{2+}/MWCNTs, 4×10^{-4} g; NaOH, 8×10^{-3} mol L^{-1}; luminol, 4×10^{-3} mol L^{-1}; H_2O_2, 4×10^{-2} mol L^{-1}; λ_{eme} 430 nm.

Table 2 Comparison of the present method with other reported methods for the determination of citalopram.

Methods	Linear range	Limit of detection (LOD)	References
HPLC	20–80 μg L^{-1}	5 μg L^{-1}	[21]
Capillary zone electrophoretic	1–20 mg L^{-1}	0.03 mg L^{-1}	[24]
Spectrofluorimetry	5.0×10^{-7}– 2.5×10^{-6} M	8.0×10^{-6}M	[29]
Spectrophotometry	10–250 μg mL^{-1}	5.2 μg mL^{-1}	[30]
Spectrophotometry	8–240 μg mL^{-1}	4.14 μg mL^{-1}	[31]
Chemiluminescence	0.2–8.0 μg mL^{-1}	$2.29 \times 10^{-5}$$\mu$g mL^{-1}	Present method

ceutical formulations. Percent recoveries were calculated and obtained in the range of 98.67–101.46 % for the pure form and 97.06–101.50 % for commercial formulations with low relative standard deviation value. The accuracy of the method was studied by the standard addition method using three different tablet brands Citalo, Lopram, and Pramcit (each containing 20 mg of citalopram). Definite amounts (0.1973, 0.4059, and 0.6081 μg mL^{-1}) of pure citalopram solution were added to tablet solutions (0.20 μg mL^{-1}) and analyzed using the described procedure.

Recoveries were calculated and obtained in the range of 97.38–101.72 % (Table 5), demonstrating the excellent accuracy of the developed method for the determination of citalopram in commercial formulations.

Table 3 Accuracy and precision of the devised method for the quantification of pure citalopram.

Amount taken /μg mL^{-1}	Amount found /μg mL^{-1}	% Recovery ± RSD
0.20	0.197	98.67 ± 0.93 %
0.40	0.406	101.46 ± 2.46 %
0.60	0.601	101.36 ± 1.11 %
Mean		100.50
± S.D.		1.58
t-test		0.443 (4.303)

Each result is the averages of separate triplicate analyses; RSD = Relative standard deviation.

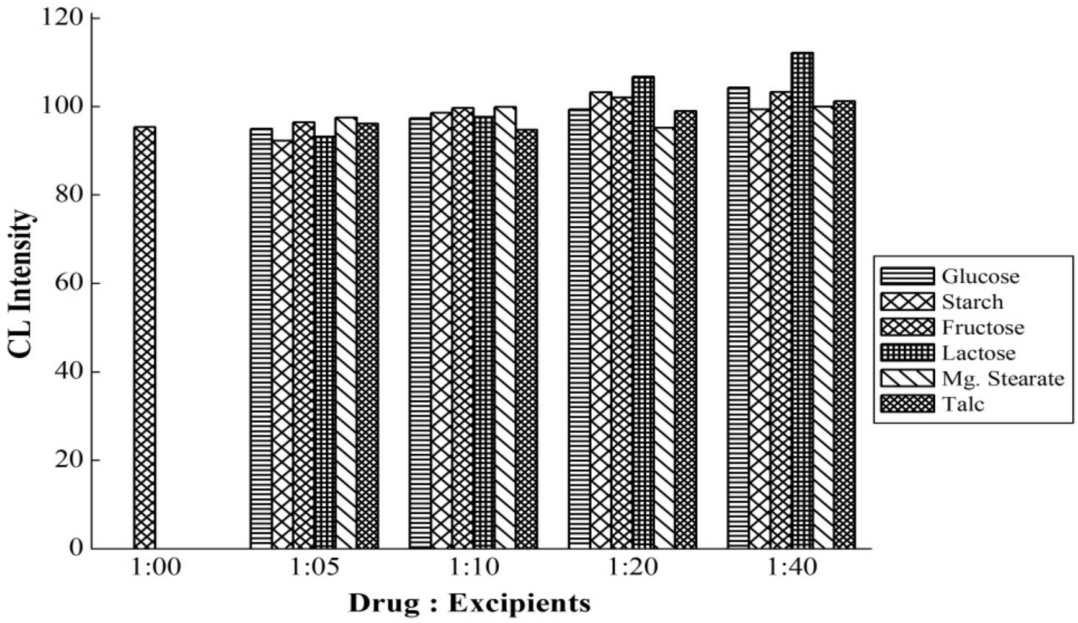

Figure 9 Effect of excipients on CL intensity.

3.7. Applicability

The proposed method was fruitfully applied to the determination of citalopram in three different pharmaceutical preparations. The results obtained agreed well with manufacturers' claims (Table 6), indicating that the method can be used to quantify citalopram levels in commercial formulations.

4. Conclusion

A simple precise accurate and sensitive method was developed for the quantification of citalopram. The method is based on the enhancement of citalopram CL of the luminol-H_2O_2-MWCNTs/Cu^{2+} system. The method has a wider linear range and lower limits of detection and quantification than other

Table 4 Evaluation of the accuracy and precision of the devised method for citalopram quantification in pharmaceutical preparations.

Pharmaceutical preparations	Amount taken /μg mL^{-1}	Amount found /μg mL^{-1}	% Recovery ± RSD
Citalo, 20 mg tablet	0.20	0.1987	99.38 ± 2.29 %
	0.40	0.3977	99.44 ± 2.35 %
	0.60	0.6085	101.42 ± 3.03 %
Lopram, 20 mg tablet	0.20	0.1941	97.06 ± 3.27 %
	0.40	0.3978	99.45 ± 1.26 %
	0.60	0.5944	99.07 ± 1.64 %
Pramcit, 20 mg tablet	0.20	0.1965	98.29 ± 3.74 %
	0.40	0.4060	101.50 ± 2.67 %
	0.60	0.5945	99.08 ± 1.83 %

Each result is the averages of separate triplicate analyses; RSD = Relative standard deviation.

Table 5 Recovery percentages for citalopram in commercial tablet formulations determined by the standard addition method.

Pharmaceutical preparation	Amount added /μg mL^{-1}	Amount found /μg mL^{-1}	% Recovery ± RSD
Citalo, 20 mg tablet	0.1973	0.1979	100.31 ± 4.52 %
	0.4058	0.4081	100.57 ± 2.42 %
	0.6081	0.6038	99.30 ± 1.28 %
Lopram, 20 mg tablet	0.1973	0.2007	101.72 ± 2.77 %
	0.4058	0.4076	100.45 ± 3.64 %
	0.6081	0.5946	97.77 ± 1.78 %
Pramcit, 20 mg tablet	0.1973	0.1992	100.97 ± 3.80 %
	0.4058	0.3952	97.38 ± 3.67 %
	0.6081	0.5972	98.20 ± 2.87 %

Each result is the averages of separate triplicate analyses; RSD = Relative standard deviation.

Table 6 Determination of citalopram active ingredients in pharmaceutical tablet preparations.

Brand name	Active ingredient /mg per tablet		
	Label value	Found value ± S.D.	% Recovery ± RSD
Citalo, 20 mg tablet	20	20.01 ± 0.234	100.06 ± 1.17 %
Lopram, 20 mg tablet	20	19.70 ± 0.257	98.52 ± 1.28 %
Pramcit, 20 mg tablet	20	19.92 ± 0.337	99.61 ± 1.69 %

Each result is the averages of separate triplicate analyses; RSD = Relative standard deviation.

described methods. In addition, it was successfully applied for the determination of citalopram in pharmaceutical preparations with good recovery and excellent reproducibility. The commonly present excipients in commercial formulations did not interfere with the analysis. Accordingly, we conclude that the developed method is appropriate for the quantification of citalopram in quality control laboratories and industrial fields.

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