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Determination of clomipramine by flow-injection analysis with acidic potassium permanganate–formic acid chemiluminescence detection

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ABSTRACT: A sensitive and simple chemiluminescent (CL) method for the determination of clomipramine has been developed by combining the flow-injection analysis (FIA) technique, which is based on the CL intensity generated from the redox reaction of potassium permanganate ($KMnO_4$)–formic acid in sulphuric acid (H_2SO_4) medium. Under the optimum conditions, the linear range for the determination of clomipramine was 0.04–4 μg/mL, with a correlation coefficient of 0.9988 ($n = 10$) and a detection limit of 0.008 μg/mL (3σ), and the relative standard deviation (RSD) for 2.0 μg/mL clomipramine ($n = 11$) is 1.26%. The proposed method has been successfully applied to the determination of the studied clomipramine in pharmaceutical preparations. The possible reaction mechanism is discussed. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: flow-injection analysis; chemiluminescence; clomipramine; potassium permanganate–formic acid

Introduction

Clomipramine hydrochloride (CMI) {3-chloro-5-[3-(dimethylamino) propyl]-10,11-dihydro-5*H*-dibenz[b,f]azepine} monohydrochloride (Fig. 1), is a typical tricyclic antidepressant (TCA) with a wide clinical spectrum, used in major depressive, panic and obsessive-compulsive disorders (OCD). In spite of new atypical drugs, such as those of the SSRI group (fluoxetine, fluvoxamine, etc.), CMI is still the reference compound in the treatment of these psychiatric disorders (1–3). Like other tricyclics, CMI inhibits noradrenalin and serotonin re-uptake in the CNS, exhibiting antimuscarinic properties as well.

Due to its therapeutical relevance, several methods have been reported for determination of CMI, including high-performance liquid chromatography (4–7), LC–MS (8), gas chromatography (9), ion-selective electrode (10), spectrophotometry (11,12) and capillary zone electrophoresis (13). However, some spectroscopic techniques are time-consuming and laborious; chromatographic techniques are slow and expensive and complicated instruments are also required. These drawbacks prevent the previously reported methods from being utilized as an official method for the analysis of pharmaceutical products.

Chemiluminescence (CL) methods have been subjected to growing interest, since they require simple and low-cost devices, providing a high versatility in the determination of a wide variety of species along with highly sensitive and wide working concentration ranges. Since no external light source is required, the absence of strong background light levels, such as those found in spectrophotometry and fluorimetry, reduces noise and leads to improved detection limits. To the best of our knowledge, no work on the determination of CMI by flow-injection analysis (FIA)–CL has been reported, except for a study of clomipramine acting as a sensitizer on the CL oxidation of sulphite by Ce(IV) with multicommutated flow system–CL (14).

In recent years, CL methods based on potassium permanganate oxidation have attracted attention and have been used to determine many drugs (15–19). The CL signal can be effectively increased in the presence of formic acid, which was also used as an enhancer in the CL reaction of potassium permanganate with some organic compounds (19–21). In this study we found that a weak CL signal was produced from the oxidation of CMI by potassium permanganate in acidic solution, while a weak CL signal was remarkably increased in the presence of formic acid. Based on these observations, a new FIA–CL method has been developed for the determination of trace amounts of CMI. This method is simpler and less expensive than the above-mentioned techniques and at the same time offers good accuracy and precision. Combined with the FIA technique, this effect provides a sensitive and convenient method for the determination of clomipramine in pharmaceutical preparations. It has been used to determine CMI in tablets and the possible CL reaction mechanism is also discussed, on the basis of UV spectra.

Experimental

Reagents and chemicals

Pure CMI powder was obtained from Nanjing Institute for Drug Control (Nanjing, China). Potassium permanganate, sulphuric acid and formic acid were purchased from Shanghai Chemical Reagent Company Ltd (Shanghai, China). All other reagents and chemicals were commercially available and of analytical reagent

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grade. All solutions were prepared with sub-boiling distilled deionized water.

The standard solution of CMI (4.0 mg/mL) was prepared by dissolving 0.4 g CMI in water and diluting to 100 mL with water, and protected from light. The stock solution (0.01 mol/L) of potassium permanganate was prepared in water (by dissolving in boiled water and filtering through glass wool) and protecting from light. A sulphuric acid solution (6.0 mol/L) and formic acid (98%, m/m) were also prepared. These standard solutions were stored in the refrigerator (4°C), and working standard solutions were prepared daily from the stock solution by appropriate dilution immediately before use.

Apparatus

The CL emission was recorded with a set of flow-injection CL analysers (IFFL-E, Xi'an Ruike Electronic equipment Corporate, Xi'an, China). A schematic diagram of the flow system used in this work is shown in Fig. 2. Two peristaltic pumps were used to deliver flow streams; one was used to deliver the flow streams of the sample (CMI) and formic acid at a flow rate of 2.5 mL/min (peristaltic 1), and the other was used to deliver the oxidant ($KMnO_4$) stream and medium (H_2SO_4) stream at a flow rate of 4.5 mL/min (peristaltic 2). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Sample injection was perfomed by a six-way injection valve fitted with a sample loop of 60 µL. The flow cell was made by coiling a 25 cm length of colourless glass tubing (1.0 mm i.d.) into a spiral disk shape and placing close to the photomultiplier tube (PMT; CR-105, Hamamatsu, Beijing, China). The CL signal was detected by the PMT with no wavelength discrimination and recorded by computer, employing the IFFL-E flow-injection CL analysis system software. The fluorescence and absorption spectra were monitored using a F-4500 fluorescence spectrometer (Hitachi, Tokyo, Japan) and a Shimadzu UV-2450 UV-visible recording spectrophotometer (Shimadzu, Kyoto, Japan), respectively.

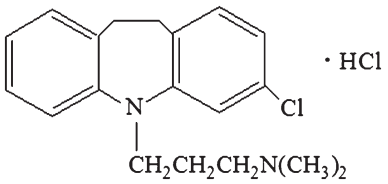

Figure 1. Chemical structures of CMI.

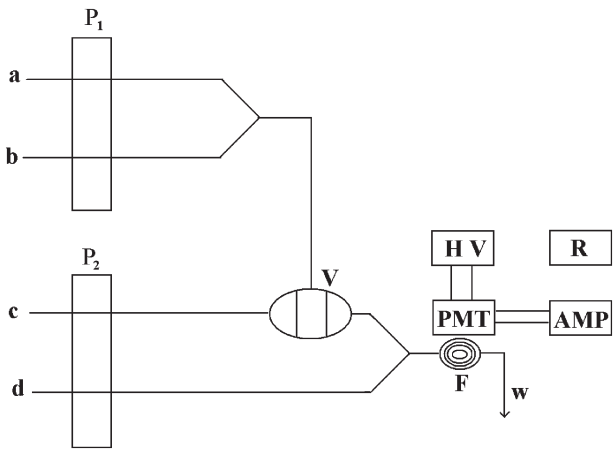

Figure 2. Schematic diagram of CL flow system. a, CMI; b, HCOOH; c, potassium permanganate; d, sulphuric acidic solution; P_1 and P_2, peristaltic pumps; V, six-way injection valve; F, chemiluminescence flow cell; PMT, photomultiplier tube; HV, negative high voltage supply; AMP, amplitude; R, recorder; W, waste solution.

Procedure

The FIA manifold was designed and fabricated as shown in Fig. 2. Solutions of CMI, formic acid, potassium permanganate and sulphuric acid were pumped continuously into the mixing element by two peristaltic pumps. The mixture of CMI with formic acid was then merged into the mixed stream of potassium permanganate and sulphuric acid by a 60 µL valve injector. The final stream was introduced into the flow CL cell. The full CL intensity vs. time curve was then recorded. The concentration of CMI was quantified by the CL intensity (peak height).

Results and discussion

Kinetic characteristics of the CL reaction

The kinetic characteristics of the CL reaction were studied by a stop-flow injection method after the baseline had been steadily recorded. Then 2.0 µg/mL clomipramine (curve a in Fig. 3) and 7.0% v/v formic acid (curve b in Fig. 3) were injected into 1.6 mol/L sulphuric acid solution containing 1.0×10^{-4} mol/L $KMnO_4$, and the CL kinetic curves were simultaneously recorded by an IFFM-E luminometer. Curve c in Fig. 3 was the CL kinetic curve obtained when the mixture solution of 2.0 µg/mL clomipramine and 7.0% formic acid was injected into 1.6 mol/L sulphuric acid solution containing 1.0×10^{-4} mol/L $KMnO_4$. It was found that the rate of the reaction was so fast that the CL intensity reached the peak maximum only 0.7 s from reagent mixing, and it took about 0.9 s for the signal decline to the base line. The results indicate that the weak CL signal of clomipramine was remarkably increased in the presence of formic acid.

Optimization of experimental conditions

To establish the optimum conditions for the determination of CMI, various parameters were investigated, using a series of univariate approaches which were performed on reagent concentration, conditions of reaction medium, reagent flow rate and injection sample volume.

The species and concentration of acids in the reaction system influence the CL intensity. Therefore, The CL emission intensities of

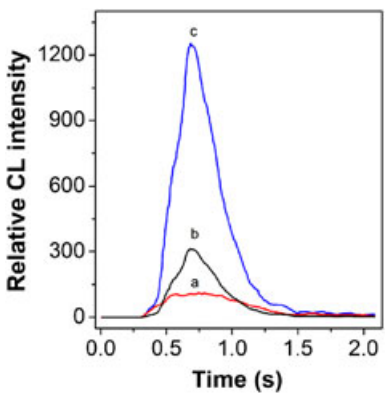

Figure 3. CL intensity–time profiles: a, $KMnO_4$ (1.0×10^{-4} mol/L) + CMI (2.0 µg/mL); b, $KMnO_4$ (1.0×10^{-4} mol/L) + HCOOH (7%); c, $KMnO_4$ (1.0×10^{-4} mol/L) + CMI (2.0 µg/mL) + HCOOH (7%). H_2SO_4, 1.6 mol/L; PMT power supply, –800 V.

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2.0 μg/mL CMI, 1.0×10^{-4} mol/L potassium permanganate and 7% v/v formic acid system were tested in the presence of five different acids, HNO_3, $HClO_4$, H_3PO_4, $H_6P_4O_{13}$ and H_2SO_4. The experimental results showed that the best signal was obtained in sulphuric acid, so sulphuric acid was selected as the optimum medium. With the increasing concentration of H_2SO_4, the CL intensity increased and reached a maximum value at 1.6 mol/L (Fig. 4). Thus, 1.6 mol/L H_2SO_4 was selected as the acidic medium for the potassium permanganate solution.

The $KMnO_4$–CMI system could only produce a weak CL emission. Various compounds, such as rhodamine B, HCHO, HCOOH, H_2O_2, Na_2SO_3, $Na_2S_2O_3$, and $Na_2S_2O_4$, were tested as sensitizers for the CL system of $KMnO_4$–CMI. It was found that only HCHO and HCOOH enhanced the CL signal for the $KMnO_4$–CMI system,which was in agreement with the results reported by Townshend and coworkers (22,23). Considering the lower background signal and fewer toxicity, formic acid was selected as a sensitizer for the CL system of $KMnO_4$–CMI. The effect of formic acid concentration upon the CL intensity was examined in the range 1.0–10.0% v/v (Fig. 5). With the increase of formic acid concentration, the CL intensity increased and reached a maximum value at 7.0%. Therefore, 7.0% was used as the optimum concentration of formic acid.

The effect of potassium permanganate concentration was examined in the range 0.2×10^{-4}–1.6×10^{-4} mol/L (Fig. 6). The CL signal increased with increasing concentration up to 1.0×10^{-4} mol/L, and began to decrease $>1.0 \times 10^{-4}$ mol/L. So, 1.0×10^{-4} mol/L potassium permanganate concentration was chosen for further experiments.

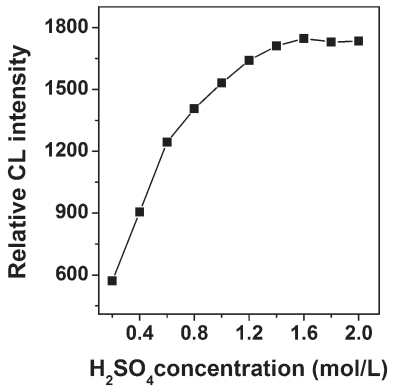

Figure 4. Effect of H_2SO_4 concentration on CL intensity of 1.0×10^{-4} mol/L $KMnO_4$–2.0 μg/mL CMI–7% v/v HCOOH.

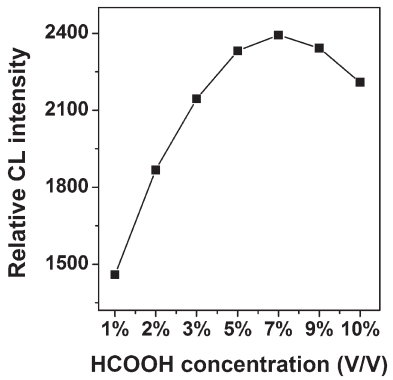

Figure 5. Effect of concentration of formic acid on the CL intensity of 1.0×10^{-4} mol/L $KMnO_4$–2.0 μg/mL CMI–1.6 mol/L H_2SO_4.

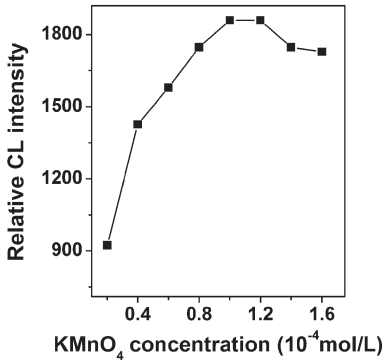

Figure 6. Effect of concentration of potassium permanganate on the CL intensity of 2.0 μg/mL CMI–1.6 mol/L H_2SO_4–7% v/v HCOOH.

Because the CL reaction was very fast, the distance between the Y-shaped mixing element and the flow cell was made to be as short as possible, and the flow rates of pumps P_1 and P_2 were studied in the range 1.0–6.0 mL/min in order to determine the maximum CL signal. When the flow rate of P_2 was 4.5 mL/min, the relative CL intensity, reproducibility of signal, peak shape and signal:noise ratio was best. Therefore, the P_2 flow rate of 4.5 mL/min was employed throughout the experiments.

Since the sampling time is sufficient, the CL signal is unchanged when the flow rates of P_1 exceeded 2.0 mL/min. Considering the reagent consumption, 2.5 mL/min was chosen as the flow rate of the sample and formic acid solution. At a flow rate of 2.5 mL/min, the determination of CMI, including sampling and washing, could be performed in 30 s, giving a sample measurement frequency of about 120 samples/h. Accordingly, the reagent consumption per analysis was about 1.5 mL.

In FIA it is necessary to optimize the injection volume to achieve the desired sensitivity. The influence of the sample injection volume on the CL intensity was tested at 40, 60, 80, 100, and 120 μL 2.0 μg/mL CMI. The highest relative CL intensity and the best signal:noise ratio were obtained when it was fixed at 60 μL. Thus, a 60 μL sample solution was injected into the carrier stream.

Analytical characteristics

Under the optimum conditions stated above, the relative CL intensity was linearly related to the concentration of CMI in the range 0.04–4.0 μg/mL. Figure 7 shows the flow-injection CL

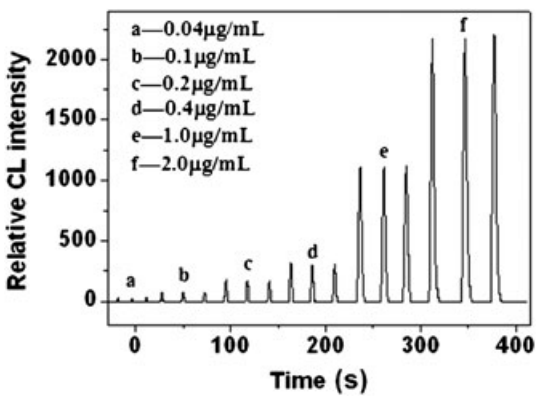

Figure 7. Typical recorder responses for the determination of CMI (a–f).

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signals for CMI. The maximum peak height increased linearly with increasing CMI concentration, with a linear regression equation of ΔI (relative units) = 15.217 c (10^{-8} g/mL) – 24.050 (r = 0.9988, n = 8). The detection limit was 0.008 μg/mL, which was calculated according to the IUPAC regulation, i.e. three times standard deviation (SD) of the blank value (3σ). It was lower than that reported on the determination of clomipramine with the multicommutated Ce(IV)-sulphite flow system–CL (14). The RSD for 11 parallel determinations of 2.0 μg/mL clomipramine was 1.26%, showing good reproducibility.

Interference

The influence of some common inorganic ions and related organic compounds was studied by the determination of 1.0 μg/mL CMI solution. The tolerance limit was taken as the amount which caused a relative error ± 5% in the peak height. The results are shown in Table 1; some ions and the studied excipients in the tablets did not interfere with the determination of CMI in this system. So, this method can be used for the determination of CMI in pharmaceutical preparations.

Determination of CMI in pharmaceutical preparations

The proposed method was successfully applied to the determination of CMI in a commercial pharmaceutical formulation. CMI tablets from different manufacturers were bought from the local market. The average content of tablets was calculated from the contents of 25 tablets; they were then finely ground, homogenized and a portion of the powder equivalent to 20 mg was weighed accurately and diluted with 50 mL water. The mixture was sonicated for 10 min and then filtered. The filtrate was diluted further with water. The sample solution was prepared by the filtrate with water in order that the concentration of clomipramine was in the working range of its determination. According to the proposed method, CMI was determined and the results are shown in Table 2. The *t*-test indicated that there were no significant differences between the results obtained by the proposed method and those obtained by the *Chinese Pharmacopoeia* method (24) at a confidence level of 95%.

Table 1. Tolerance to different substances in the determination of 1.0 μg/mL clomipramine

Species added	Maximum tolerable ratio (relative error ± 5%)
Zn^{2+}, Mg^{2+}, K^{+}, Na^{+}, Ca^{2+}, NH_4^{+}, SO_4^{2-}, NO_3^{-}, Cu^{2+}, Al^{3+}, PO_4^{3-}	500
Ni^{2+}, Pb^{2+}	200
Sucrose, starch, CH_3CHOO^{-}	100
Glucose, lactose^{+}	50
Citric acid, tartaric acid, $C_2O_4^{2-}$, Fe^{3+}	20
Ascorbic acid	5

Possible CL mechanism

Absorbance spectra of 1.0×10^{-4} mol/L $KMnO_4$ solutions were examined in order to obtain more information about the enhanced CL mechanism. A 1.0×10^{-4} mol/L $KMnO_4$ solution (curve a in Fig. 8), a mixture of 1.0×10^{-4} mol/L $KMnO_4$ with 50.0 mg/L clomipramine (curve b in Fig. 8) and a mixture of 1.0×10^{-4} mol/L $KMnO_4$ with 50.0 mg/L clomipramine and 2.0% formic acid (curve c in Fig. 8) were respectively scanned. The absorbance peaks of $KMnO_4$ at 524 nm and 545 nm significantly decreased in the presence of clomipramine and more remarkably decreased in the presence of clomipramine and formic acid simultaneously. These results indicated that an oxidation–reduction reaction occurred between $KMnO_4$ and clomipramine and energy was released, while formic acid evidently enhanced the reaction.

The CL reactions of polyhydroxyl (25–29) and polyamine (22,23,30,31) compounds with oxidizing agents have been extensively investigated, and singlet excited molecular oxygen species was proposed as the possible emitter. Formic acid enhances permanganate CL from a variety of analytes and, while it might simply accelerate the oxidation reaction rate, amine reductants with a tertiary amine, such as clomipramine, could make a specific contribution to the production of the CL signal. It has previously been suggested (20,22,29,32–34) that $KMnO_4$ could react with some reductants in the presence of formaldehyde or formic acid to produce $^1O_2$ $^1O_2$ ($^1\Delta g$ $^1\Delta g$), a dimeric oxygen molecule in the singlet state, which emits light as it relaxes to $^3O_2$ ($^3\Sigma g$), a triplet state oxygen. Thus, it is possible that the singlet excited molecular oxygen species is an emitter in the present system, formed by the transfer of energy from oxidized clomipramine to dissolved oxygen. Based on the above discussions, a possible mechanism for this process is:

$$MnO_4^- + H^+ + CH_2O_2 + \text{clomipramine} \rightarrow H_2O + Mn^{2+} + \text{oxidized clomipramine intermediate}$$
$$^3O_2(^3\Sigma g) + \text{oxidized clomipramine intermediate} \rightarrow {}^1O_2(^1\Delta g) + \text{oxidized clomipramine product}$$
$$2\,{}^1O_2(^1\Delta g) \rightarrow {}^1O_2^1O_2(^1\Delta g\,{}^1\Delta g)$$
$${}^1O_2^1O_2(^1\Delta g\,{}^1\Delta g) \rightarrow 2\,{}^3O_2(^3\Sigma g) + h\nu$$

Table 2. Analytical results for the samples (mg/tablet)

Sample	Nominal content	Proposed method	RSD (%, n = 5)	Pharmacopoeia method	RSD (%, n = 5)
H19994048	25	25.37	1.26	24.78	1.56
H31020406	25	25.25	1.82	25.22	1.38
H31021551	25	24.46	1.39	25.36	1.07

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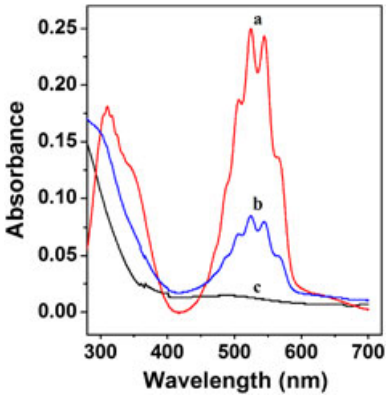

Figure 8. UV spectra of: a, $KMnO_4$ (1.0×10^{-4} mol/L) + H_2SO_4 (1.6 mol/L); b, $KMnO_4$ (1.0×10^{-4}mol/L) + H_2SO_4 (1.6 mol/L) + CMI (50 μg/mL); c, $KMnO_4$ (1.0×10^{-4} mol/L) + H_2SO_4 (1.6 mol/L) + CMI (50 μg/mL) + HCOOH (2%).

Conclusions

A sensitive and simple flow-injection CL method was proposed for the determination of CMI based on the acidic $KMnO_4$–formic acid system. The experimental conditions for the CL reaction were optimized and the analytical characteristics for the determination of CMI are presented here. The possible CL reaction mechanism has also been discussed on the basis of UV spectra. The proposed method is simple, rapid, inexpensive and sensitive for the determination of CMI in tablets. This method is practical and valuable in clinical and biochemical laboratories for the determination of clomipramine.

Acknowledgements

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