Scientific Research

Flow Injection Determination of Tramadol Based on Its Sensitizing Effect on the Chemiluminescent Reaction of Permanganate-Sulfite

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Abstract

In this paper, a novel chemiluminescence (CL) method for the determination of tramadol has been developed by combining the flow injection technique and its sensitizing effect on the weak CL reaction between sulfite and acidic KMnO$_4$. A mechanism for the CL reaction has been proposed on the basis of fluorescent and CL spectra. Under the optimized conditions, the proposed method allows the measurement of tramadol hydrochloride over the range of 0.04 - 4 μg/mL with a correlation coefficient of 0.9995 ($n = 8$) and a detection limit of 0.01 μg/mL (3σ), and the relative standard deviation for 2.0 μg/mL tramadol ($n = 11$) is 2.1%. The utility of this method was demonstrated by determining tramadol hydrochloride in tablets and injections.

1. Introduction

Tramadol[(±)trans-2-(dimethylaminomethyl)-1-(3-methoy-phenyl)-cyclohexanol] (**Figure 1**) hydrochloride is a centrally acting analgesic agent which possesses an analgetic action with a potency ranging between weak opioids and morphine [1,2]. It is mainly metabolized by liver and essentially excreted by the kidney [2,3]. Clinical studies have shown that respiratory depression, pronounced opioid side-effect profile and analgetic tolerance hardly happen after repeated administration are observed with tramadol [4]. Therefore, it is successfully used since 1977 for the relief of short-term and long-term pains which may be induced by the different illnesses or traumas in the world [5].

Various methods have been reported for the determination of tramadol hydrochloride in biological fluids and in pharmaceutical preparations, including HPLC [6-11], HPTLC [12], GC [13,14], capillary electrophoresis(CE) [15,16], spectrophotometry [17-19], voltammetry [20] and potentiometry [21]. Each of the above methods had its own merits and disadvantages, for example , some of them were accurate and selective, but involved expensive instrumentation and were time consuming, needed preconcentration before determination because of lacking high sensitive detector; some were inherent simple but

have low sensitivity . Therefore, it is very important to develop a rapid, simple, sensitive and accurate method for the determination of tramadol in order to obtain optimal therapeutic concentrations for quality assurance in pharmaceutical preparations.

Flow-injection chemilluminescence(CL) methods are powerful analytical techniques that have been frequently used for the analysis of pharmaceuticals in recent years and have excellent sensitivity, wide linear dynamic range, a high degree of reproducibility, which requires relatively simple and inexpensive instrumentation [22-25]. To the best of our knowledge, up to the present time, nothing has been published concerning the determination of tramadol with a flow injection CL method.
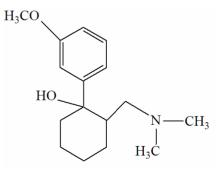

Figure 1. Chemical structures of tramadol.

In order to develop a simple, rapid, and sensitive method for the determination of tramadol hydrochloride in both pure form and its pharmaceutical preparations, this work studied the sensitizing effect of tramadol on the CL intensity emitted from the reaction of sulfite with acidic $KMnO_4$. A method for tramadol detection is proposed on the basis of this sensitizing effect. This method is simple and less expensive than the above-mentioned techniques and at the same time offers good accuracy and precision. Combining with flow injection technique, this effect provides a sensitive and convenient method for the determination of tramadol in pharmaceutical preparations. It has been used to determine tramadol hydrochloride in tablets and injections successfully. The possible CL reaction mechanism has also been discussed on the basis of CL spectra.

2. Experimental

2.1. Apparatus

The CL emission was recorded with a set of flow-injection CL analyzer (IFFL-E, Xi'an Ruike Electronic equipment Corporate, Xi'an, China). The schematic diagram of the flow-injection CL analytical system was shown in **Figure 2**. Two peristaltic pumps were used to deliver flow streams. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. A flow cell that positioned in front of the detection window of the photomultiplier tube (PMT) (CR-105, Hamamatsu, Beijing, China) was a 25 cm length of spiral glass tubing (1.0 mm i.d.) and the distance between injection valve and flow cell was about 10 cm. Sample solution of tramadol hydrochloride was injected into Na_2SO_3 carrier stream using a six-way injection valve equipped with a 80 μL sample loop, merged with $KMnO_4$ solution stream, and then generated CL emission in the flow cell. The CL signal was detected by the PMT with no wavelength discrimination and recorded with computer employing an IFFL-E flow-injection CL analysis system software. The fluorescence and absorption spectra were monitored using a F-2500 fluorescence spectrometer (Hitachi, Tokyo, Japan) and a Shimadzu UV-2450 UV—visible recording spectrophotometer (Shimadzu, Kyoto, Japan), respectively. The CL spectrum was obtained with a series interference filters by the static method. The filters were inserted between the sample cuvette and the photomultiplier tube (PMT).

2.2. Reagents and Chemicals

Pure powders of tramadol hydrochloride was obtained from Nanjing Institute for Drug Control (Nanjing, China). $KMnO_4$ and Na_2SO_3 were purchased from Shanghai Chemical Reagent Limited Company (Shanghai, China). All other reagents and chemicals were commercially available and of analytical reagent grade. All solutions were prepared with sub-boiling distilled deionized water.

The standard solution of tramadol hydrochloride (1.000 mg/ml) was prepared by dissolving 0.0500 g tramadol hydrochloride with water and diluting to 50mL with water, stored in the refrigerator (4°C) and diluted as required daily. The Na_2SO_3 solution (1.0×10^{-2} mol/L) was prepared by dissolving 0.1260 g Na_2SO_3 with 100 mL water daily and kept in a dark place. Potassium permanganate solution (1.0×10^{-2} mol/L) was prepared by dissolving 1.58 g of $KMnO_4$ in 1 L of boiled water and filtering through glass wool.

2.3. Procedure

As shown in **Figure 2**, flow lines (a-d) were connected with tramadol hydrochloride solution, Na_2SO_3 solution, water and $KMnO_4$ with H_2SO_4 solution, respectively. Flow rate was set at 4.2 mL/min for the $KMnO_4$ solution and water, respectively; sample and Na_2SO_3 solution at 2.5 mL/min. Pumps were started to wash the whole system until a stable blank signal was recorded. A 80μL mixture of tramadol and Na_2SO_3 was injected into the carrier stream (H_2O), which was merged with acidic $KMnO_4$ solution for producing CL signal. The concentration of tramadol hydrochloride was quantified by the CL intensity (peak height).
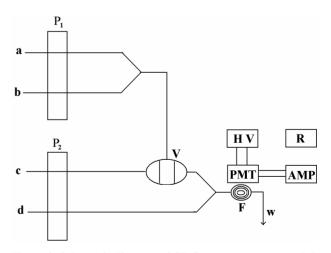

Figure 2. Schematic diagram of CL flow system. a, tramadol; b, sodium sulfite solution; c, carrier(H_2O); d, $KMnO_4$; P_1 and P_2, peristaltic pump; V, six-way injection valve; F, CL flow cell; PMT, photomultiplier tube; HV, negative high voltage supply; AMP, amplitude; R, recorder; W, waste solution.

3. Results and Discussion

3.1. The Kinetic Characteristics of the CL Reaction

The kinetic characteristics of the CL reaction were studied by a stop-flow injection method after the baseline was steadily recorded. Then 3.0×10^{-3} mol/L Na_2SO_3 was injected into 0.08 mol/L sulphuric acid solution containing 1.0×10^{-4} mol/L $KMnO_4$ and the CL kinetic curve was simultaneously recorded by IFFM-E luminometer (see **Figure 3**, dashed line). Solid line in **Figure 3** was the CL kinetic curve obtained when the mixture solution of 2.0 μg/mL tramadol and 3.0×10^{-3} mol/L Na_2SO_3 were injected into 0.6 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 0.8 s for the signal decline to the base line. The results indicate that the CL signal was remarkably increased in the presence of tramadol.

3.2. Optimization of the Experimental Conditions

To establish the optimum conditions for the determination of tramadol hydrochloride, 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 kinds and concentration of acids in the reaction system influence the CL intensity. Therefore, six different acids including HCl, HNO_3, CH_3COOH, H_3PO_4, $H_6P_4O_{13}$ or H_2SO_4 of different concentration in the range of 0.02 - 0.2 mol/L were tested, respectively. The results showed 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 0.08 mol/L (**Figure 4**). Thus, 0.08 mol/L H_2SO_4 was selected as the acidic medium for the $KMnO_4$ solution.

The effect of $KMnO_4$ concentration on CL intensity was examined in the range of 0.2×10^{-4} to 1.6×10^{-4} mol/L (**Figure 5**). With the increasing concentration of $KMnO_4$, the CL intensity increased and reached a flat value at 1.0×10^{-4} mol/L. Thus, 1.0×10^{-4} mol/L $KMnO_4$ concentration was chosen for further experiments.

The effect of Na_2SO_3 concentration upon the CL intensity was examined within the range of 1.0×10^{-3} to 4.0×10^{-3} mol/L (**Figure 6**). With the increase of Na_2SO_3 concentration, the CL intensity increased and reached a maximum value at 3.0×10^{-3} mol/L. Therefore,

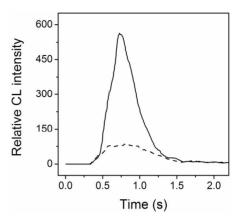3.0×10^{-3} mol/L was used as the optimum concentration of Na_2SO_3.

Figure 3. CL intensity-time profiles of $KMnO_4$-Na_2SO_3 reaction (dashed line) and $KMnO_4$-Na_2SO_3-tramadol hydrochloride reaction(solid line).
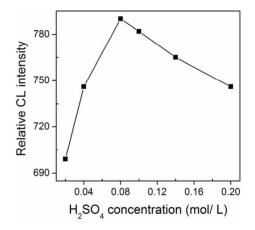

Figure 4. Effect of H_2SO_4 concentration on CL intensity of 1.0×10^{-4} mol/L $KMnO_4$ + 1.0 μg/mL tramadol hydrochloride + 3.0×10^{-3} mol/L Na_2SO_3.
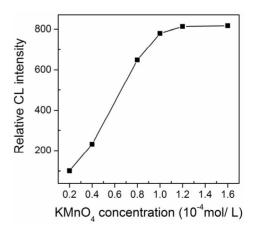

Figure 5. Effect of $KMnO_4$ concentration in 0.08 mol/L H_2SO_4 on CL intensity of 1.0 μg/mL tramadol hydrochloride + 3.0×10^{-3} mol/L Na_2SO_3.
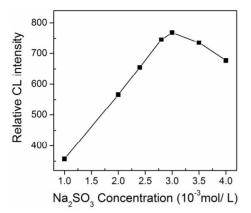
Figure 6. Effect of Na₂SO₃ concentration on CL intensity of 1.0 μg/mL tramadol hydrochloride + 1.0 × 10⁻⁴ mol/L KMnO₄ concentration in 0.08 mol/L H₂SO₄.

Because the proposed CL reaction was very fast, the distance between Y-shaped mixing element and the flow cell was made to be as short as possible, and the flow rate of pump P₂ was studied in the range of 1.0 to 6.0 mL/min in order to determine the maximum of the CL signal. When the flow rate was 4.2 mL/min, the relative CL intensity, reproducibility of signal, peak shape, and ratio of signal to noise was the best. Therefore, the flow rate of 4.2 mL/min was employed throughout the experiment.

At the flow rate of 4.2 mL/min, the determination of tramadol hydrochloride, including sampling and washing, could be performed in 20s, giving a sample measurement frequency of about 180 samples per hour. Thus, it was decided to supply the KMnO₄ solution and carrier stream at 4.2 mL/min, respectively. Considering the reagent consumption, 2.5 mL/min was chosen as the flow rate of the sample and Na₂SO₃ solution.

In flow injection analysis, 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 of 2.0 μg/mL tramadol hydrochloride. The biggest relative CL intensity and the best ratio of signal to noise were obtained when it was fixed between 80 and 100 μL. Thus, a 80 μL sample solution was injected into the carrier stream.

3.3. Analytical Characteristics of Tramadol Hydrochloride

Under the optimum conditions mentioned above, the relative CL intensity was linearly related to the concentration of tramadol hydrochloride from 0.04 to 4 μg/mL. **Figure 7** shows the flow injection chemiluminescence signals for tramadol. The maximum peak height increased

linearly with the increasing of tramadol concentration, with a linear regression equation of ΔI (relative units) = 754.1 c (μg/mL) + 121.6 (r = 0.9995, n = 8). The detection limit was 0.01 μg/ml, which was calculated according to IUPAC regulation that is three times of standard deviation of blank value (3σ). The relative standard deviation for 11 parallel determinations of 1.0 μg/mL tramadol was 2.1%, showing a good reproducibility.

3.4. Interferences Experiments

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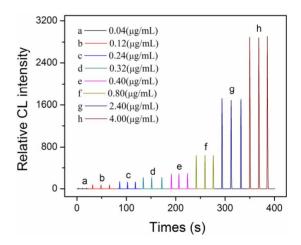

Figure 7. Typical recorder response for the determination of tramadol (a-h).

Table1. Tolerance to different substances in the determination of 1.0 μg/mL tramadol hydrochloride.

Species added	Maximum tolerable ratio
Na^+, K^+, Mg^{2+}, Zn^{2+}, Al^{3+}, NO_3^-	500
Ca^{2+}, Cu^{2+}	100
starch, Fe^{3+}, Pb^{2+}	50
glucose, sucrose, CH_3COO^-	20
Citric acid, Ni^{2+}, Cr^{3+}	10
ascorbic acid, tartaric acid	5

3.5. Determination of Tramadol Hydrochloride in Pharmaceutical Preparations

The proposed method was successfully applied to the determination of tramadol in a commercial pharmaceutical formulation. The tramadol hydrochloride tablets and injections 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 which was equivalent to 20 mg was weighed accurately and diluted with 50 mL water. The mixture was sonicated for 10 min and then filtrated. The filtrate was diluted further with water. On the contrary, the injection of tramadol hydrochloride needed not any pre-treatment. The sample solution was prepared by directly diluting the injection and the filtrate with water in order that the concentration of tramadol was in the working range of its determination. According to the proposed method, tramadol hydrochloride was determined and the results were 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 at confidence level of 95%.

3.6. Possible CL Mechanism

The reaction of sulphite with strong oxidants such as $KMnO_4$ in acid media is accompanied by a weak CL that has been reported extensively [26,27], it is proposed that the excited SO_2^* is the emitter and the emission spectrum is between 450 and 600 nm [28,29]. This weak CL reaction can be sensitized by not only several fluorescent, which is the energy-transfer mechanism[22,30] but also non-fluorescent compounds such as 3-cyclohexylaminopropane sulfonic acid (CAPS) [31], ibuprofen[32] and iproniazid [33] etc. Although the causes of the sensitizing action of these non-fluorescent compounds are different from each other, the sensitizing effect of CAPS is attribute to the presence of the cyclohexyl ring [31,34].

On the one hand the molecule of tramadol hydrochloride (**Figure 1**) contains a cyclohexyl ring, on the other hand the CL spectrum of $KMnO_4$-Na_2SO_3-H_2SO_4 system in the presence and absence of tramadol hydrochloride showed similar emission profile extending from 450 to 600 nm (**Figure 8**), which was measured by means of a series of interference filters. According to the suggested reports, the emitter is the excited sulfur dioxide. Therefore, it may be concluded that tramadol hydrochloride played a role of an enhancer in the reaction.

The fluorescence spectra of tramadol solution were scanned in the range 250 - 700 nm, using a F-2500 fluorescence spectrometer (Hitachi, Tokyo, Japan). Since the fluorescence spectrum of tramadol (λ_{ex} = 200 nm, λ_{em} = 300 nm) was not identical with the CL spectrum obtained, and the reaction product of $KMnO_4$ with tramadol was a non-fluorescent compound, an energy-transfer mechanism was excluded.

The absorbance spectra of $KMnO_4$-H_2SO_4 solution and $KMnO_4$-H_2SO_4-tramadol solution were scanned (**Figure 9**). The absorbance peaks of $KMnO_4$ at 524 nm and 545 nm decreased in the presence of tramadol and the decreased quantity was related to the concentration of tramadol. According to the suggestion reported in [32], the mechanism of tramadol-$KMnO_4$-Na_2SO_3-H_2SO_4 system could be expressed as follows:

$$MnO_4^- + HSO_3^- \rightarrow HSO_3^\cdot + MnO_4^{2-}$$

$$2HSO_3^\cdot \rightarrow S_2O_6^{2-} + 2H^+$$

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2^*$$

$$KMnO_4 + tramadol \rightarrow \ products + energy$$

$$SO_2 + energy \ \rightarrow \ SO_2^*$$

$$SO_2^* \rightarrow SO_2 + h\nu$$

Table 2. Results of determinationof tramadol hydrochloride in injections and tablets.

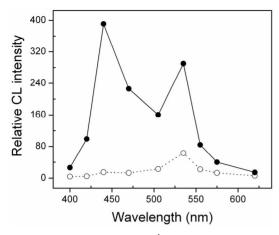| Samples	Nominal Content	Proposed Method	RSD (%, n = 5)	Pharmacopoeia Method	RSD (%, n = 5)
Injection 1(H20023786)	50 mg/2mL	49.7 ± 0.4 (mg/2mL)	2.0	49.8 ± 0.2	1.6
Injection 2 (J20020086)	100 mg/2mL	99.9 ± 0.5 (mg/2mL)	1.8	99.7 ± 0.4	1.5
Tablet 1 (H20033330)	100 mg/tablet	99.7 ± 0.6 (mg/2mL)	1.9	99.6 ± 0.3	1.4
Tablet 2 (H10960043)	50 mg/tablet	49.6 ± 0.7 (mg/2mL)	2.1	49.7 ± 0.5	1.5

Figure 8. CL spectra of 1.0×10^{-4} mol/LKMnO$_4$ + 3.0×10^{-3} mol/LNa$_2$SO$_3$ (dashed line) and 20.0 μg/mL tramadol hydrochloride + 1.0×10^{-4} mol/LKMnO$_4$ + 3.0×10^{-3} mol/L Na$_2$SO$_3$ (solid line).
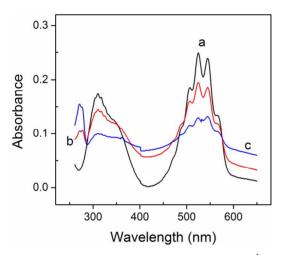

Figure 9. Absorbance spectra of: (a) 1.0×10^{-4} mol/L KMnO$_4$ + 0.08 mol/L H$_2$SO$_4$; (b) 1.0×10^{-4} mol/L KMnO$_4$ + 0.08 mol/L H$_2$SO$_4$ + 10.0 μg/mL tramadol; and (c) 1.0×10^{-4} mol/L KMnO$_4$ + 0.08 mol/L H$_2$SO$_4$ + 20.0 μg/mL tramadol.

The CL of KMnO$_4$-Na$_2$SO$_3$ reaction was very weak. The CL signal was remarkably increased in the presence of tramadol. Therefore, it can be concluded that tramadol played a role of an enhancer in the reaction.

4. Conclusions

A new flow injection CL method was proposed for the determination of tramadol hydrochloride based on the KMnO$_4$-sodium sulfite system. The experimental conditions affecting the CL reaction were optimized and the analytical characteristics for the determination of tramadol hydrochloride are presented here. The possible CL reaction mechanism has also been discussed. The proposed method has a simple, rapid, inexpensive and high sensi-

tivity for the determination of tramadol hydrochloride in tablets. This method is practical and valuable in clinical and biochemical laboratories for the determination of tramadol.

5. Acknowledgements

6. References

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