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Determination of ethamsylate in pharmaceutical preparations based on an auto-oxidation chemiluminescence reaction

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Abstract

Strong chemiluminescence emission has been observed by mixing alkaline hydrolytic products of ethamsylate with Tween 80 in acidic rhodamine 6G solution. This phenomenon has been utilized to design a flow-injection chemiluminescence method for the determination of ethamsylate in a pharmaceutical preparation. Under the optimum conditions, the proposed procedure has a linear range between 0.05 and 2.0 μg ml^{-1}, with a detection limit of 0.02 μg ml^{-1} for ethamsylate. The method was applied to the determination of ethamsylate in pharmaceutical preparations. The possible mechanism of this chemiluminescence reaction was proposed.

Keywords: Ethamsylate; Dissolved oxygen; Tween 80; Rhodamine 6G; Chemiluminescence

1. Introduction

Ethamsylate, or 2,5-dihydroxybenzenesulfonic acid with diethylamine, is a systemic haemostatic agent that has been suggested for the prophylaxis and control of hemorrhages due to the rapture of small blood vessels in surgery and in clinical conditions [1–3]. Although this compound has been extensively applied for clinical purposes, only few papers have been published for the determination of ethamsylate in recent years. Hassan et al. proposed a potentiometric method for ethamsylate determination [4], the detection limit was around 0.1 mmol l^{-1} ethamsylate. Another method was based on the suppression of chemiluminescence (CL) intensity by ethamsylate in the luminol-hypochlorite system [5], but the method is relatively complex because the hypochlorite used in the method was generated by electrolysis and the conditions for the determination were difficult to control: in addition its linear range was rather narrow.

Our preliminary experiment showed that weak CL emission could be produced by mixing the alkaline hydrolytic products of ethamsylate with Tween 80 in acidic solution. The CL intensity

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could be greatly sensitized by adding rhodamine 6G to the solution. This phenomenon allowed us to develop a sensitive method for the determination of ethamsylate in a pharmaceutical preparation by using a simple flow-injection system. As the CL emission was produced by the reaction of dissolved oxygen with ethamsylate in Tween 80 medium sensitized by rhodamine 6G, the reaction was therefore named as the auto-oxidation CL reaction [6] in the present paper.

2. Experimental

2.1. Apparatus

The schematic diagram of flow-injection (FI) analyzer for the CL determination of ethamsylate is shown in Fig. 1. The FI system consisted of one peristaltic pump and a six-way valve. The peristaltic pump was used to deliver the sample solution of ethamsylate and the mixture reagent solution of Tween 80 and rhodamine 6G in acidic medium at a flow rate of 3.0 ml min^{-1}. A six-way valve with a sample loop of 200 μl was automatically operated by a computer to inject sample into the streams of the reagent solutions. The distance between the valve and the flow cell was 10 cm. The CL emission was transformed into an electrical signal by a photomultipler tube operated at -900 V. The data was collected and recorded by the computer.

2.2. Reagents and solutions

All reagents were of analytical-reagent grade unless specified otherwise; doubly distilled water was used for the preparation of solutions. Ethamsylate was purchased from the Chinese Institute for the Control of Pharmaceutical and Biomedical Products (Beijing, China). Ethamsylate injection and tablets were purchaded from Beijing Pharmaceutical (Beijing, China). The tablet is a mixture of ethamsylate and amylum, containing 250 mg of ethamsylate per tablet. The injection is an aqueous solution of ethamsylate with a concentration of ethamsylate of 250 mg/2 ml. Tween 80 was obtained from Dalian pharmaceutical company (Dalian, China). Rhodamine 6G was obtained from Baker (Phillipsburg, NJ). An ethamsylate stock solution (1 mg ml^{-1} in water) was kept in a brown flask and stored in a refrigerator. Standard solutions of ethamsylate in the range 0.05–2 μg ml^{-1} were prepared by appropriate dilution of the stock standard solution in water. Tween 80 solutions was prepared with distilled water. A total of 0.02 mol l^{-1} rhodamine 6G stock solution was prepared by dissolving 0.479 g rhodamine 6G in 50 ml water and dilution to give the working solution of 0.05 mmol l^{-1}. The CL intensities versus ethamsylate concentrations were used for the construction of a calibration curve.

2.3. Sample analysis

The tablets and injection of ethamsylate were purchased locally. The tablet is a mixture of ethamsylate and amylum, containing 250 mg of ethamsylate per tablet. The injection is the aqueous solution of ethamsylate with a concentration of ethamsylate 250 mg/2 ml.

Average tablet weight was calculated by weighing ten tablets. An accurate weight portion of the homogenized powder containing 50 mg of ethamsylate was transferred to a 50 ml volumetric brown flask and diluted to volume with doubly distilled water. The powder was completely disintegrated by a mechanical shaker and the solution was filtered. Working solutions were prepared by appropriate dilution of the concentrated sample solutions with 4 mmol l^{-1} NaOH solution. A total of 2 ml of the ethamsylate injection solution was also diluted appropriately with water contain-

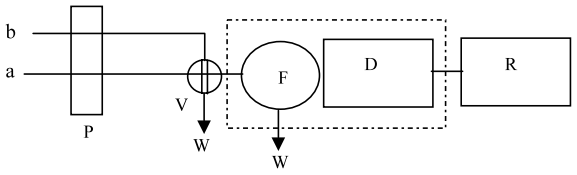


Fig. 1. Schematic diagram of flow the system for the determination of ethamsylate. (a) Reagent stream containing 0.5% Tween 80, 0.5mmol l^{-1} rhodamine 6G and 0.02 mol l^{-1} H_2SO_4; (b) ethamsylate solution in 4 mmol l^{-1} sodium hydroxide; P, peristaltic pump; V, injection valve; F, flow cell; D, Detector; R, computer, W, waste.

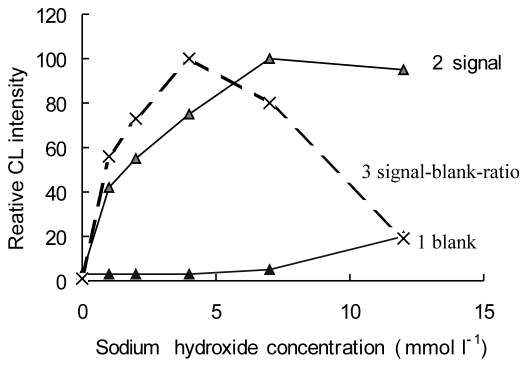


Fig. 2. Effect of NaOH concentration on CL intensity. Rhodamine 6G 0.05 mmol l^{-1}; H_2SO_4 0.02 mol l^{-1}; Tween 80 0.5% (w/v); ethamsylate 0.5 μg ml^{-1}.

ing 4 mmol l^{-1} NaOH solution and the final sample concentration was in the working range.

3. Results and discussion

3.1. Optimization

3.1.1. Effect of pH values

In the preliminary experiment, no CL intensity was observed when ethamsylate was mixed with Tween 80 and rhodamine solutions in acidic medium. However, after hydrolysis of ethamsylate in alkaline medium, strong CL emission could be produced by mixing hydrolyzed ethamsylate with Tween 80 in acidic rhodamine 6G solution, indicating that the hydrolytic product of ethamsylate, but not ethamsylate itself, could react with these reagents to produce CL emission. The preliminary experiment also showed that the pH values played an important role for the CL reaction. The pH effect was therefore examined in the first place for two reasons. First, the effect of pH on ethamsylate hydrolysis was examined. It was found that an alkaline medium was necessary to hydrolyze ethamsylate. However, the background signal was increased at the same time along the increase of NaOH concentration. To compromise the signal to background ratio, 4 mmol l^{-1} NaOH concentration was chosen as optimal condition. Fig. 2 shows the effect of NaOH concentration on the hydrolysis of ethamsylate from 0.0 to 12.0 mmol l^{-1}.

Although the ethamsylate hydrolysis should be carried out under alkaline condition, the CL emission could only be generated in acidic medium in the present study. Therefore, the effect of sulfuric acid concentration was examined over the range of 0.0–0.3 mol l^{-1}. The results are shown in Fig. 3. The maximum emission was obtained at 0.005 mol l^{-1} sulfuric acid. However, if the signal/blank ratio was taken into account, the preferred concentration of sulfuric acid was 0.02 mol l^{-1}.

3.1.2. Effect of Tween 80 concentration

This (organized) surfactant is currently utilized for improving CL quantum efficiencies or energy-transfer efficiencies by many authors. This potential was therefore examined in the preliminary experiment of this study so as to enhance the CL emission for ethamsylate determination. A distinct enhancement of CL emission was observed in the present study. Without Tween 80 in the solution, almost no CL signal could be detected by the injection of ethamsylate into the reagent stream (shown in Fig. 1). However, strong CL emission was subsequently detected when Tween 80 was added to the reagent stream, indicating that the surfactant plays an important role for the CL reaction. In order to investigate which kind of surfactants possessed the enhancing effect upon the present CL reaction, different types of surfactants (cationic, anionic and neutral) were studied.

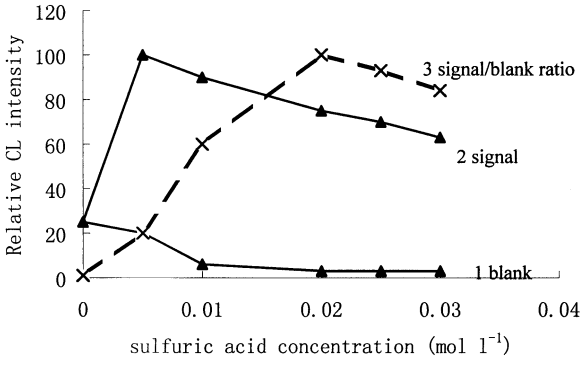


Fig. 3. Effect of sulfuric acid concentration on CL intensity. Rhodamine 6G 0.05 mmol l^{-1}; Tween 80 0.5% (w/v); ethamsylate 0.5 μg ml^{-1} in 4 mmol l^{-1} sodium hydroxide.

Table 1
Effect of surfactants on CL emission

Surfactants[a]	Relative CL intensity
None	1.0
Triton X-100	1.0
Polyethylene glycol-400	1.8
Glycol	1.1
Tween 85	44.0
Tween 80	100.0
SDS	1.0
Cetylpyridinium bromide	1.0
Cetuldimethylbenzylammonium chloride	1.0

[a] Concentration: 0.5% (w/v).

As can be seen from Table 1, apart from the Tween series, all other types of surfactants could not enhance CL emission. The effect of Tween 80 concentration was studied so as to maximize the CL signal. As shown in Fig. 4, the maximum CL signal could be obtained using a concentration of 0.5% (w/v) Tween 80.

3.1.3. Effect of rhodamine 6G concentration

The fluorescers were considered as energy transfer reagents in the CL reaction as these reagents produced remarkable increases in CL emission. Therefore a variety of fluorescent compounds were examined to achieve a maximum CL signal. From the results listed in Table 2, apparently most compounds cannot significantly produce CL emission except for the rhodamine series. Rhodamine 6G was found the best to enhance CL emission. The effect of rhodamine 6G over the concentration range 0.0–0.2 mmol l^{-1} was examined and the results are shown in Fig. 5. It can be seen that the higher CL signal was obtained with 0.1 mmol l^{-1} rhodamine 6G, but the optimum signal/background ratio was obtained with 0.05 mmol l^{-1} rhodamine 6G. This concentration was therefore used in further experiments.

Table 2
Effect of fluorescers on CL emission

Fluorescer	Concentration	Relative CL intensity
None		1.0
Rhodamine B	0.1 mmol l^{-1}	66.0
Rhodamine 6G	0.1 mmol l^{-1}	100.0
Butyl-rhodamine B	0.1 mmol l^{-1}	57.0
Pheno-safranine	0.02% (g ml^{-1})	1.0
Safranin T	0.02% (g ml^{-1})	1.0
Acridine yellow	0.02% (g ml^{-1})	1.0
Quinie	0.1 mmol l^{-1}	1.0

3.1.4. Effect of flow rates

The flow rate of reagent stream containing Tween 80 and rhodamine 6G in sulfuric acid was optimized in order to obtain satisfactory CL emission. It was found that the CL intensity increased with the increase in flow rate, indicating a fast dynamic process of this reaction. The results are illustrated in Fig. 6. As a compromise between

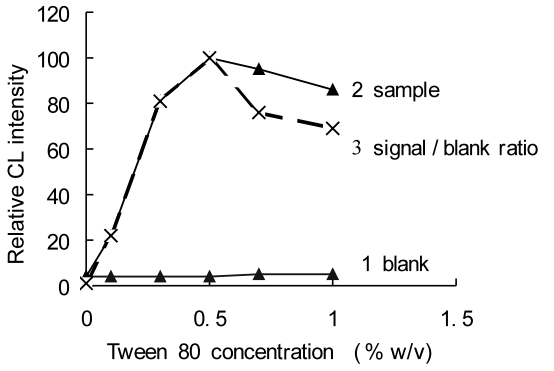


Fig. 4. Effect of Tween 80 concentration on CL intensity. Rhodamine 6G, 0.05 mmol l^{-1}; H_2SO_4 0.02 mol l^{-1}; ethamsylate, 0.5 μg ml^{-1} in 4 mmol l^{-1} sodium hydroxide.

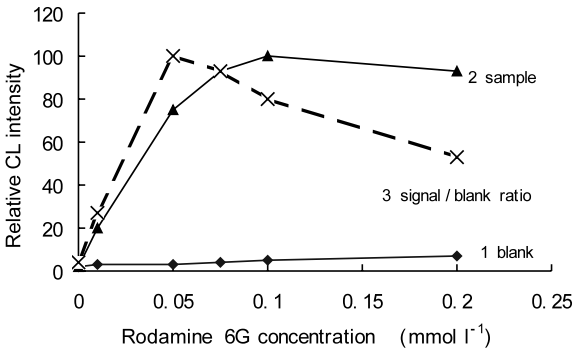


Fig. 5. Effect of rhodamine 6G concentration on CL intensity. H_2SO_4, 0.02 mol l^{-1}; Tween 80, 0.5% (w/v); ethamsylate, 0.5 μg ml^{-1} in 4 mmol l^{-1} sodium hydroxide.

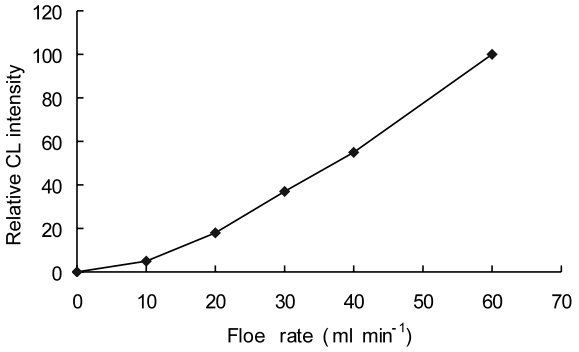


Fig. 6. Effect of the flow rate on CL intensity. Rhodamine 6G, 0.05 mmol l^{-1}; Tween 80, 0.5% (w/v); H_2SO_4, 0.02 mol l^{-1}; ethamsylate, 0.5 μg ml^{-1} in 4 mmol l^{-1} sodium hydroxide.

reagent consumption and CL intensity, 3.0 ml min^{-1} of flow rate was recommended.

3.2. Interference

The effect of common components used as excipients was examined by adding certain amounts of each excipient to sample solutions containing 0.5 μg ml^{-1} ethamsylate. The tested excipients included fructose, lactose, maltose, magnesium stearate, sucrose, amylum, glucose, dextrin, $H_2PO_4^-$ and HCO_3^-, etc. The insoluble materials, if any, were filtered before measurement. The results are listed in Table 3. When the concentrations of excipients were of 1.0 mg ml^{-1}, the relative error caused by these substances was < 4%.

Table 3
Interference by some excipients on the CL intensity

Excipient (1.0 mg ml^{-1})	Relative CL intensity	Relative error (%)
None	100.0	
Fructose	98.0	2.0
Glucose	101.0	1.0
Lactose	100.0	0.0
Maltose	97.0	3.0
Sucrose	102.0	2.0
Amylum	100.0	0.0
Magnesium stearate	98.0	2.0
Dextrin	99.0	1.0
$H_2PO_4^-$	97.0	3.0
HCO_3^-	97.0	3.0

3.3. Calibration and detection limit

Under the optimum conditions described above, the calibration of emission intensity versus ethamsylate concentration was linear at the range of 0.05–2 μg ml^{-1}. The regression equation was $I = 973.5C - 57.5$ ($r = 0.998$, $n = 7$), where I is the emission intensity (mv) and the C is the concentration of ethamsylate (μg ml^{-1}). The detection limit of ethamsylate was 0.02 μg ml^{-1}. The relative standard deviations ($n = 6$) were 2.6% for 0.1 μg ml^{-1} and 3.9% for 1.0 μg ml^{-1}, respectively.

3.4. Sample analysis

In order to evaluate the validity of the proposed method for the determination of ethamsylate in pharmaceutical formulations, the recovery test was carried out by adding known amounts of ethamsylate standard to injection and to tablets. As shown in Table 4, the recoveries were 99.0–103.2%, indicating that the method is reliable for the quantitation of ethamsylate in pharmaceutical preparations. The reliability of the proposed method was also evaluated by comparing the results with those obtained from UV spectrometry. No significant differences were observed between the both methods.

3.5. Possible mechanism

In order to understand the mechanism of this CL reaction, the hydrolytic products were identified in the present work by using mass spectrometry. It was found that ethamsylate was converted into 2,5-dihydroxybenesulfonate and diethylamine after treatment in alkaline medium as shown below:

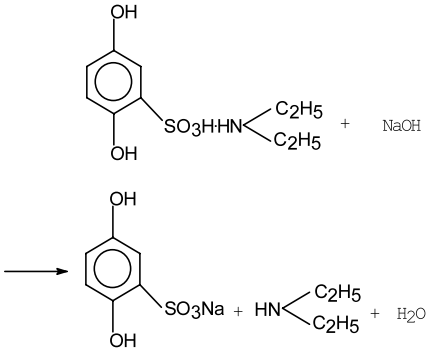
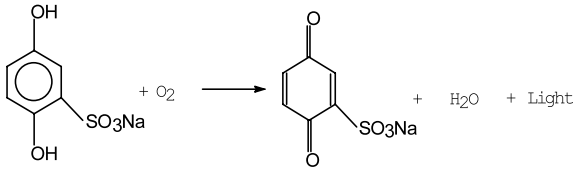


Table 4
Determination of ethamsylate in pharmaceutical formulations

Sample	Amount (mg)			Added (mg)	Recovered (mg)	Recovery (%)
	Label	Proposed method Found ± S.D.[a]	UV method Found ± SD[a]			
Injection®	250.0	251.0 ± 0.5	249.0 ± 0.3	100.0	353.1	102.1
				50.0	300.8	99.6
Tablet®	250.0	248.9 ± 0.3	249.5 ± 0.4	125.0	372.6	99.0
				50.0	300.5	103.2

[a] S.D., standard deviation ($n = 8$).

Strong CL emission could be generated when 2,5-dihydroxybenesulfonate reacted with dissolved oxygen in Tween 80 medium. When dissolved oxygen is removed from the medium by using nitrogen, the CL emission was greatly decreased. Based on this fact, we deduced that the possible mechanism of this CL reaction would be that 2,5-dihydroxybenesulfonate is oxidized by dissolved oxygen as shown below. The reaction would be sensitized by rhodamine 6G in the presence of Tween 80 surfactant micelles. Further study is obviously needed to confirm this deduction so as to understand this newly developed CL reaction without adding oxidant to the reacting system.



4. Conclusion

A method for the determination of ethamsylate based on the auto-oxidation of ethamsylate by dissolved oxygen in acid rhodamine 6G and Tween 80 surfactant micelles is proposed in the present study. Compared to the methods for the determination of ethamsylate reported in literature, the present method offers advantages of simplicity and sensitivity. It should also be noted that the mechanism of the present method is different from current CL methods, in which the added oxidants such as Ce(IV), permanganate, iodine and hydrogen peroxide have to be involved, hence the selectivity in the proposed reaction is therefore improved.

Further work on the physiochemical nature of the suggested reaction, including possible interferences is in progress.

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