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Short communication

On-line chemiluminescence determination of mitoxantrone by capillary electrophoresis

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ABSTRACT

A novel capillary electrophoresis (CE) with chemiluminescence (CL) detection method for the determination of mitoxantrone (MTX) has been developed, which based on the CL reaction of potassium ferricyanide with luminol in sodium hydroxide medium sensitized by MTX. Under optimum analytical conditions, MTX is determined over the range of 7.0×10^{-8}–1.0×10^{-6} M with a detection limit of 1.0×10^{-8} M. The relative standard deviation (RSD) was 3.7%, 2.6% and 3.0% for 7.0×10^{-8}, 5.0×10^{-7} and 1.0×10^{-6} M MTX ($n = 11$), respectively. In laboratory-built CE–CL apparatus, the proposed method has been applied to determination of MTX in commercial drug and spiked in human urine and plasma with satisfactory results.

1. Introduction

Mitoxantrone (MTX, Fig. 1), a synthetic anthracenedione derivative, is a clinically well-established anticancer agent, which shows high efficacy in breast cancer, acute leukemia and non-Hodgkin's lymphoma [1,2]. Thus, its determination in biological media is very important and requires the development of highly sensitive and precise methods for clinical assay. Nowadays, MTX has been determined by HPLC [3–7], electrochemical assays [8–10], radioimmunoassay [11,12], resonance Raman scattering (RRS) [13], resonance Rayleigh scattering (RRS) [14] and chemiluminescence (CL) [15]. Thereinto, several methods mentioned above can achieve a very low limit of detection (LOD) of concentration, however, the required sample volumes were relatively large, and this led to an unsatisfying absolute LOD. Furthermore, some of the above method had no separation capability, which made it incapable of handling complex systems containing various components such as urine and plasma.

Capillary electrophoresis (CE) has developed into an attractive analytical tool for the separation of various types of complex mixtures [16,17]. CE is known to possess high resolution, relatively short analysis time, low operational costs, instrumentation simplicity, and compatibility with small sample volumes. CL detection, due to its simple optical system and low background nature [18], has become a candidate for a new and sensitive CE detection scheme [19,20]. In this work, it was observed that MTX could enhance the CL emission of luminol–$K_3Fe(CN)_6$ in basic solution and the enhancement degree was proportional to the content of MTX. Hence, a novel CE–CL system was established for the determination of the MTX. The proposed method exhibits high sensitivity and has been successfully applied to detect MTX in injection, serum and urine samples solution. To the best of our knowledge, no CL method coupled with CE has been previously reported for the determination of MTX in the open literature.

2. Experimental

2.1. Chemicals and reagents

Luminol was obtained from Shannxi Normal University (Xi'an, China). Mitoxantrone (MTX) was purchased from Shanghai Institute of Pharmaceutical Industry (Shanghai, China). All the other chemicals used in this work were of analytical grade. Ultra pure water (Germany) was used to prepare all solutions.

The 0.01 M luminol stock solution was prepared by dissolving 0.177 g luminol in 0.1 M NaOH solution, and diluting to 100 mL with water. The 0.1 M borate buffer stock solution and 0.01 M $K_3[Fe(CN)_6]$ stock solution was prepared in water. The 1.0 mM MTX stock solution was prepared by dissolving 0.0444 g MTX in 100 mL water. Working solutions were freshly prepared by diluting MTX stock solution with water. All solutions were stored in a refrigerator at 4 °C.

The running buffer solution contained 8.0 mM borate solution and 0.6 mM luminol solution. The post-column CL solution was the

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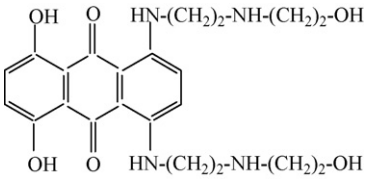

Fig. 1. The structure of MTX.

0.1 mM $K_3[Fe(CN)_6]$ solution and 0.1 M NaOH solution. All solutions were filtered through a 0.45 μm membrane filter.

2.2. CE–CL apparatus and CE conditions

All the experiments were performed using a laboratory-built CE–CL system (Fig. 2). Briefly, a 0–30 kV high-voltage power supply (Institute of Atom Nucleus, Shanghai, China) provided the separation voltage. A 50 cm × 50 μm i.d. uncoated fused silica capillary (Hebei Optical Fiber, China) was used for the separation. The polyimide on 5 cm end section of the separation capillary was burned and removed. After etching with HF for 1 h, this end of separation capillary was inserted into the 530 μm i.d reaction capillary. A four-way plexiglass joint held a separation capillary and a reaction capillary in place. The CL solution was delivered by gravity through a reagent capillary of 320 μm i.d. into a tee. The grounding electrode was put in one joint of the tee. The outlet of the reaction capillary was 2 cm lower than the other end to make the solution flow out the reaction capillary more easily and quickly. The CL solution flowed down to the detection window, which was made by burning 1 cm of the polyimide of the reaction capillary and was placed in front of the photomultiplier tube (PMT). The CL emission was collected with a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) and then recorded using a computer with BPCL software. The whole CL detection system was held in a large light-tight box to exclude stray light.

In this study, the new capillary was preconditioned by flushing with 1 M NaOH for 30 min before the first use. The separation capillary was filled with running buffer while the four-way joint and reaction capillary were filled with CL solution. After each run, the separation capillary was treated with running buffer for 5 min. The sample was introduced by hydrodynamically for 10 s at a height difference of 15 cm. The applied voltage was 20 kV.

3. Results and discussion

3.1. Optimization of CL conditions

The CL reaction of luminol with $K_3[Fe(CN)_6]$ in NaOH medium can be sensitized by the MTX. From the structure of MTX, MTX contains two phenolic hydroxyl groups, making this drug easily being oxidized in solution. So, the CL reaction of luminol–$K_3Fe(CN)_6$–MXT system could be described as follows [21,22]:

$$\text{Luminol} + K_3Fe(CN)_6 + NaOH \rightarrow \text{luminol radical}$$

$$MTX + K_3Fe(CN)_6 + NaOH \rightarrow [MTX]^* \qquad [MTX]^* + O_2 \rightarrow O_2{}^*$$

$$\text{Luminolradical} + O_2{}^* \rightarrow [\text{aminophatalate}]^*$$

$$[\text{aminophatalate}]^* \rightarrow \text{aminophatalate} + h\nu$$

where $[MTX]^*$ indicates the intermediate in the oxidation reaction of the MTX and $O_2{}^*$ indicates the superoxide radical. The luminophor of the system is luminol.

To maximize the sensitivity of CL detection, the CL conditions were examined and several parameters such as the concentrations of luminol, $K_3[Fe(CN)_6]$, borate and NaOH, the applied voltage were optimized. In these experiments, a 5.0×10^{-7} M of MTX solution was injected into the CE–CL system and the CL intensity (peak height) was recorded.

3.1.1. Effect of NaOH concentration on the CL intensity

It is well known that the characteristics of the CL signal are pH-dependent and are sensitive to its changes. In this work, owing to luminol reacts with $K_3[Fe(CN)_6]$ to produces CL in alkaline condition, the NaOH solution was selected as the reaction medium to control the pH value of reaction. Therefore, NaOH concentration was the first parameter evaluated for its effect on the analytical response. It was observed that higher CL signal and better repeatability could be obtained when NaOH solution was added into the system by post-column as the oxidizer medium than into luminol solution. The effect of NaOH concentration on the CL reaction was examined in the 0.01–0.2 M. Finally, 0.1 M of NaOH was employed because it gave higher CL signal.

3.1.2. Effect of luminol concentration on the CL intensity

The effect of the concentration of luminol in the running buffer on the CL intensity was investigated over the range of 0.1–1.0 mM. The results (Fig. 3A) indicate that the CL intensity increased gradually with increasing luminol concentration up to 0.6 mM, where maximum CL intensity was reached, and further increasing the luminol concentration results in a decrease in CL intensity. Thus, the luminol concentration of 0.6 mM was chosen for sequent research work.

3.1.3. Effect of $K_3[Fe(CN)_6]$ concentration on the CL intensity

The effect of $K_3[Fe(CN)_6]$ concentration on CL intensity is shown in Fig. 3B. As can be seen from Fig. 3B, the CL intensity first increased tremendously and then decreased with the increase in $K_3[Fe(CN)_6]$ concentration. The maximum CL intensity was obtained when the concentration was at 0.1 mM. So 0.1 mM of $K_3[Fe(CN)_6]$ concentration was accepted as an optimum concentration in this study.

3.1.4. Effect of borate concentration

In the CE–CL detection system, MTX migrated in the separation capillary, where it mixed with luminol included in the running buffer. The running buffer concentration affects directly the migration rate of MTX and luminol in capillary, which cause the change of CL intensity. To examine the change, several borate solutions concentration ranges from 1.0 to 15.0 mM were tested. The results indicate that maximum CL signal was obtained when the concentration was 8.0 mM. Therefore, the concentration of 8.0 mM borate buffer (pH 8.2) was used in this work.

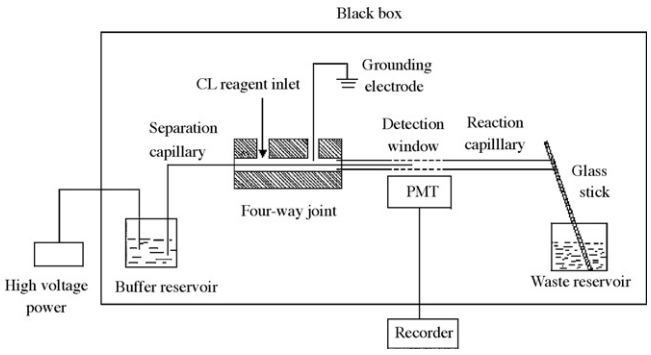

Fig. 2. Schematic diagram of the CE–CL system for the determination of MTX.

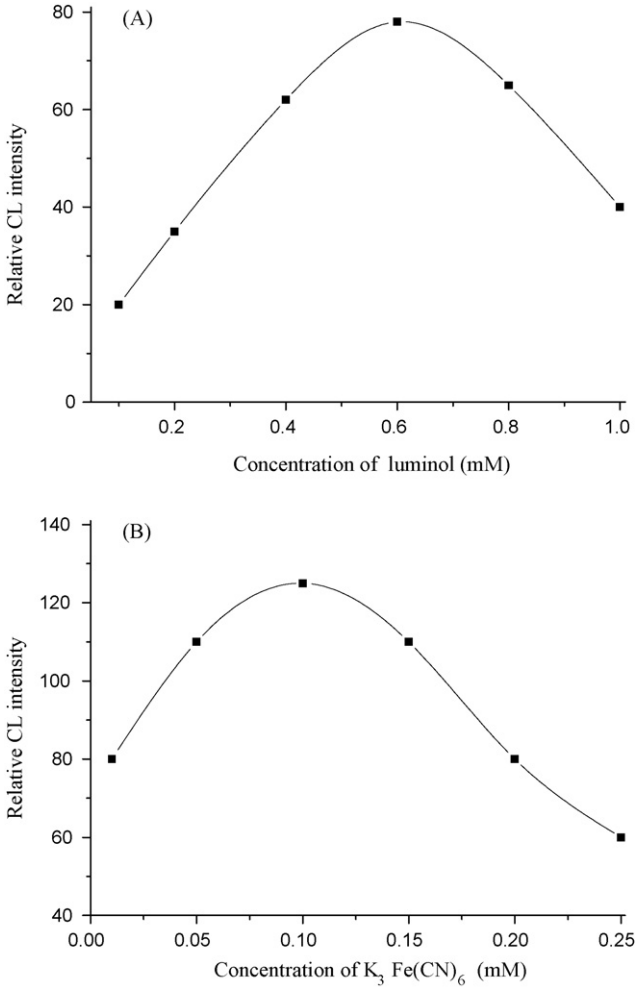

Fig. 3. Effect of concentration of luminol (A) and $K_3Fe(CN)_6$ (B) on the CL intensity, respectively. Electrophoresis electrolyte was 0.6 mM luminol in 8.0 mM borate buffer. The CL solution was 1.0 mM $K_3[Fe(CN)_6]$ in 0.1 M NaOH solution. Capillary was 50 μm i.d. × 50 cm effective length. Applied voltage was 20 kV.

3.1.5. Effect of the applied voltage

Dependence of CL intensity on applied voltage was examined over the voltage ranges of 12–22 kV, and the experiment results showed that the applied voltage of 20 kV is the optimal one to obtain higher CL intensity.

After a careful study on the effects of above several parameters, the CL conditions for the determination of MTX were selected as following: 20 kV applied voltage; running buffer consisted of 8.0 mM borate (pH 8.2) and 0.6 mM luminol; the oxidizer solution consisted of 0.1 mM $K_3[Fe(CN)_6]$ in 0.1 M NaOH solution. Under optimum conditions, the electrophoregram for MTX was shown in Fig. 4A.

3.2. Method validation

The CE–CL method was evaluated in terms of the response linearity, limit of detection and reproducibility. The calibration curve was linear over the concentrations range of from 7.0×10^{-8} to 1.0×10^{-6} M, and the limit of detection (S/N = 3) for MTX was estimated to be 1.0×10^{-8} M. The regression equation of calibration curve for MTX was $\Delta I = 31.64c + 365.62$ ($r = 0.9968$), where ΔI is the peak high and c is the concentration of MTX in μM. The reproducibility was investigated by injecting a 1.0×10^{-6}, 5.0×10^{-7} and 7.0×10^{-8} M MTX standard solution 11 times and recording the peak high. The reproducibility of the method was demonstrated by the mean RSD. The RSD calculated from peak height was 3.0%, 2.5% and 3.7%, respectively.

3.3. Analytical application

3.3.1. Determination of MXT in injection

Without any pretreatment, 2.0 mL injection sample was diluted to 10 mL with water and further diluted to the working range of the determination of MTX, then analyzed according to the procedure described above. The analytical results for commercial MTX drug were compared with those declared on the injection label, i.e. the amount declared was 2.0 mL/phial. The amounts found were between 2.05 and 1.98 mg/tablet. The RSD of reproducibility were ranged between 2.2% and 3.3%.

3.3.2. Determination of MXT in plasma

Fresh plasma sample (taken from healthy people) and suitable amounts of acetonitrile were mixed thoroughly and centrifuged at 5000 rpm for 10 min to separate proteins. The supernatant was transferred into another centrifuge tube and dried with an N_2 stream. The residue was dissolved in water. 0.5 mL aliquot of the supernatant fluid was pipetted into a 5.0 mL volumetric flask for the determination of MXT. The RSD and recovery were tested by using the standard addition method. The results are listed in Table 1.

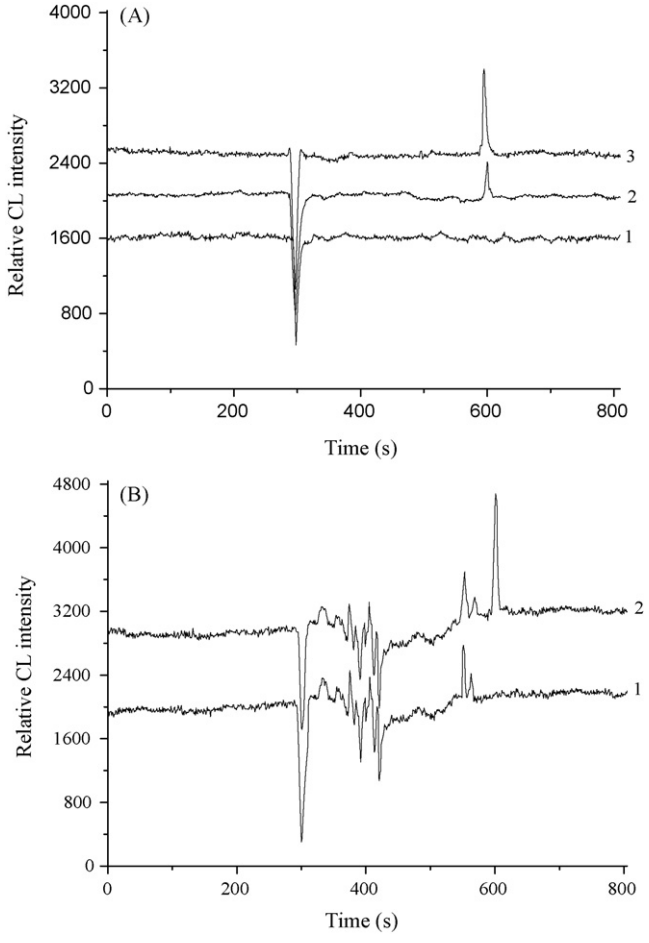

Fig. 4. Electropherograms of the standard MTX solution (A) at 0.0 M (trace 1), 1.0×10^{-7} M (trace 2) and 5.0×10^{-7} M (trace 3); electropherograms of human urine samples (B) at blank urine sample (trace 1) and urine sample added with 3.0×10^{-7} M MTX (trace 1). Other conditions were as in Fig. 3.

Table 1
Results for the determination of MTX in urine and plasma ($n = 5$).

Sample	Found (M)	Added (10^{-7} M)	Total found (10^{-7} M)	Recovery (%)	RSD 1 (%)	RSD 2 (%)
Urine 1	ND	7.0	6.95	99.3	2.27	2.30
Urine 2	ND	3.0	3.07	102.3	2.35	2.28
Urine 3	ND	1.0	1.03	103.0	1.98	2.02
Plasma 1	ND	7.0	7.20	102.9	2.34	2.23
Plasma 2	ND	3.0	3.0	100.0	1.85	1.90
Plasma 3	ND	1.0	9.8	98.0	3.06	3.05

ND: not detected; RSD 1: RSD of repeatability; RSD 2: RSD of reproducibility.

3.3.3. Determination of MXT in urine

Fresh urine sample (healthy people) was collected in a vial. After centrifugation for 10 min at 5000 rpm, 1.0 mL of the supernatant fluid was pipetted into a 10.0 mL volumetric flask for the determination of MXT. The RSD and recovery were examined by using the standard addition method. The results are shown in Table 1.

It can be seen from Table 1 that the method has a good repeatability (RSD are from 1.98% to 3.06%), reproducibility (RSD are from 1.90% to 3.05%) and accuracy (recovery are from 98.0% to 103.0%). This assay was a novel method for monitoring hemic-medical and uric-medical concentration of MXT in clinical pharmacology and for the pharmacokinetics study, also provided valuable criterion for use of anthracycline anticancer drugs rationally, safely and effectively.

Fig. 4B shows the electropherograms of urine samples without and with the addition of the standard MTX solution, which made it possible to identify MTX in the sample and to evaluate its concentration. As can be seen from Fig. 4B, there was not the peak observed across the migration time of MTX in the electropherogram of blank urine (trace 1). After the urine samples were added with MTX, it was found that a peak for the determination of MTX was observed in the electropherogram of urine added with MTX (trace 2), which indicated that none of endogenous compounds in urine would interfere with the determination of MTX. The plasma electropherograms also showed that there were no peaks observed across the elution time in the electropherogram of blank plasma.

4. Conclusion

The post-column direct CL detection with CE has been used for the determination of MTX as a simple, fast, sensitive and selective method. Quantitative measurements of MTX in human urine and plasma have been demonstrated, and no interference was found from samples matrix, which proves that the CE–CL method may be used in clinical analysis. In comparison with other possible methods, the proposed procedure is selective and sensitive. The simple direct determination of MTX is a good alternative to existing HPLC methods giving a short analysis time, low cost and minimal organic waste.

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