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A Novel Sensitized Chemiluminescence Flow Injection System for the Determination of Adriamycin and Mitomycin

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A novel chemiluminescence (CL) method was established for two anticancer drugs, adriamycin (ADM) and mitomycin (MMC), based on potassium permanganate oxidation in the presence of formaldehyde. The sensitized CL emission mechanism was developed by comparing the fluorescence emission with CL spectra. Illuminant was the singlet state bi-molecule oxygen, $^1O_2{}^1O_2$ ($^1\triangle_g{}^1\triangle_g$), from $^1O_2$ ($^1\triangle_g$) which was produced in the reaction system, and emitted CL spectra at 639 nm or 649 nm. The presence of formaldehyde may accelerate the generation of $^1O_2$ ($^1\triangle_g$) and sensitized CL emission. The optimum conditions for CL emission were investigated and optimized. The relationships between the relative CL intensity and the concentration of the studied analytes found to be linear. The detection limit was 3×10^{-8} g ml^{-1} for ADM and 3×10^{-9} g ml^{-1} for MMC. The relative standard deviations are 2.2% and 1.8% for determinations of ADM at 2.0×10^{-6} g ml^{-1} and MMC at 2.0×10^{-7} g ml^{-1}, respectively. The proposed sensitized CL system was successfully applied to the determination of ADM and MMC in their injections with satisfactory results.

Keywords: Chemiluminescence, Potassium permanganate, Formaldehyde, Adriamycin, Mitomycin

INTRODUCTION

Adriamycin (ADM) is one of the most powerful anticancer drugs in the field of cancer chemotherapy, and presently used for the clinical treatment of a broad range of human malignancies, such as leukemia, and cancer of the breast, endometrium, ovary, stomach, multiple myeloma, lymphomas, and lung [1-5]. Mitomycin C (MMC) is an antineoplastic drug widely used in clinical chemotherapy. Tomasz *et al*. studied isolation and structure of a covalent cross-link adduct between MMC and DNA. Mitomycin C produces intra- and interstrand cross-linking between residues in DNA, resulting in the production of "bulky lesions" [6]. MMC is frequently used in combined chemotherapy with 5-fluorouracil drugs [7]. Chemical structure of the two anticancer drugs is shown in Fig. 1.

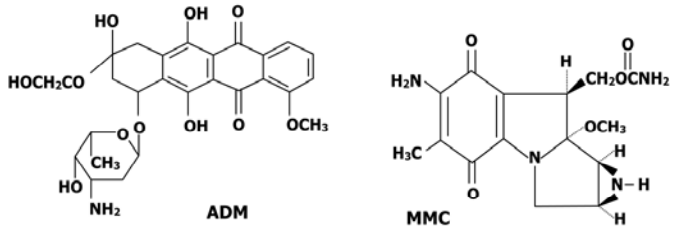

Fig. 1. Chemical structure of the anticancer drugs.

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Recently, clinical research has shown that some metabolites and derivatives of ADM and MMC have stronger bioactivity than the anticancer drugs. Quality control of drug dosage, its monitoring in biological fluids, and research of drug's metabolism and action are important analytical tasks. Therefore it is necessary to establish sensitive analytical techniques for the drug analysis.

HPLC-UV detection was listed in State Drug Standard by the Pharmacopoeia Commission of the People′s Republic of China [8]. Several methods have been used for the determination of ADM in human cells by using HPLC-UV detection [9], in urine samples by electrochemical analysis [10,11], and in tumor tissue by a fluorospectrophotometric method with a detection limit of 1×10^{-7} g ml^{-1} [12]. HPLC-UV method was also listed in the Chinese Pharmacopoeia for MMC determination in its injection form [13]. HPLC method has been used to determine MMC in lipiodol emulsion [14], human plasma [15,16] and ocular tissues [17,18]. A voltammetric method was used for the determination of MMC in urine [19]. Recently a sensitive method has been developed for the assay of traces of MMC in hen aqueous humour samples by HPLC with electrospray ionization mass spectrometric detection with a detection limit of 2×10^{-9} g ml^{-1} [20]. However, the reported methods were complicated, the sensitivity was low or the analytical conditions were harsh or the instrument was expensive.

Chemiluminescence (CL) is becoming a powerful analytical tool with widespread application in various fields owing to its high sensitivity, wide dynamic range and simple instrumentation. A critical review on the use of acidic potassium permanganate for CL generation during the oxidation of both organic compounds and inorganic species has been published [21]. Several studies on the generation of very sensitive CL emission by oxidation of hydroxyl-containing drugs with $KMnO_4$ in acidic medium have been reported [22-25]. It was also reported that formaldehyde could enhance the CL emission intensity of such system, which laid a foundation of using HCHO as a sensitized agent in CL analysis, and stimulated researchers to explore its application as a sensitized agent in other fields. Chen *et al*. used potassium permanganate-formaldehyde system for the CL determination of melatonin and some of its derivatives [26]. A time-resolved CL method was also proposed for the CL determination of hydralazine in pharmaceuticals using phosphoric-acidified $KMnO_4$ as oxidant [27]. The analytical utility of soluble manganese(IV) as a CL reagent in the presence of formaldehyde was developed [28,29]. This CL system was used to determine melatonin and some of its derivatives [30]. A CL method was developed for the determination of naftopidil based on potassium permanganate oxidation in the presence of formaldehyde or formic acid [31]. A novel flow injection CL method for the fast, simple and sensitive determination of three estrogens has been investigated based on the CL reaction of the studied estrogens with formaldehyde and potassium permanganate in sulfuric acid medium [32]. Although CL method has been used for the analysis of a variety of pharmaceuticals, there is few reports on CL method for the study and detection of anticancer drugs.

The main purpose of this paper is to develop a new sensitized CL system based on the use of acidified $KMnO_4$ as oxidant in the presence of formaldehyde for the determination of two anticancer drugs. The proposed sensitized CL system has been successfully applied to the determination of ADM and MMC in their injections with satisfactory results. A corresponding CL mechanism has also been developed.

EXPERIMENTAL

Chemicals

All chemicals used were of analytical-reagent grade. Deionized water was used throughout. ADM and MMC were supplied by Institute of Medicinal Biotechnology (Beijing, China). ADM stock standard solution (5.0×10^{-4} g ml^{-1}) was prepared by dissolving 25.0 mg ADM, followed by diluting with deionized water to 50 ml. MMC stock standard solution (5.0×10^{-4} g ml^{-1}) was prepared by dissolving 25.0 mg MMC in 2 ml 0.1 M NaOH and diluting with deionized water to 50 ml. More dilute solutions were freshly prepared by appropriate dilutionof the stock solutions with deionized water. A stock solution of $KMnO_4$ (5×10^{-2} M) was prepared by dissolving a weighed amount of $KMnO_4$ in water and diluting to the volume. A 2.5% (V/V) of HCOH solution and a 3 M of HNO_3 solution were daily prepared.

Apparatus

All fluorescence spectra were recorded with a RF-5301PC

spectrofluorometer (Shimadzu, Japan). The FI system used was a MPI-B-FI-CL analysis system (Xi'an Remex Electronic science-tech Co. Ltd., Xi'an, China) consisting of two peristaltic pumps working at a constant flow rate (30 rpm) and a six-way injection valve with a sample loop (120 µl), which is automatically operated by a computer equipped with a software for operation system of MPI-B flow injection analysis (Fig. 2). The flow cell was a twisted glass tube in order to produce a large surface area exposed to the adjacent photomultiplier tube (PMT) (Hamamatsu, Japan). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system.

Because there is no reaction between $KMnO_4$ solution and HNO_3 solution，the two solutions were merged and used as carrier. The mixture flowed into a flow cell through a three-way pipe. Then a mixture of sample and HCOH solution was injected from a sample valve. The immediately produced CL signal was recorded.

Sample Preparation

The powder contents of at least 10 ADM injection bottles (Haizheng Pharmaceuticals Ltd Co., Zhejiang, China) or 20 MMC injection bottles (Hengrui Pharmaceuticals Ltd Co., Jiangsu, China) were thoroughly mixed and weighed to obtain the mean mass per bottle. An accurately weighed portion of each homogenized powder containing 10 mg ADM or MMC was dissolved with water in a small beaker. The solution was filtered and the residue was washed with water several times, then transferred into a 25 ml calibrated flask and diluted to the mark with water. Working solutions were prepared by appropriate dilution of this sample solution so that the final analytical concentration was within the working range.

Procedure

As shown in Fig. 2, all solutions were continuously pumped into the manifold. A 120 µl mixture of ADM (or MMC) and HCOH solution was injected into a mixed stream of $KMnO_4$ and HNO_3 solutions. The mixed solution was transferred into the CL flow cell, which immediately resulted to an intensive CL signal. The calibration graphs were constructed by plotting the intensity (peak height) of the CL signal versus the analyte concentration.

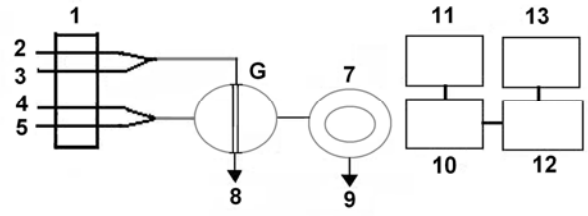

Fig. 2. Schematic diagram of flow injection CL analysis system: (1) peristaltic pump, (2) analyte solution, (3) HCOH solution, (4) $KMnO_4$ solution, (5) HNO_3 solution, (6) sampling inlet valve, (7) flowing cell, (8,9) waste, (10) photomultiplier tube, (11) high voltage, (12) amplifier, (13) recorder.

RESULTS AND DISCUSSION

Effect of Sample Volume and Flow Rate

As shown in Fig. 2, when the mixed solution flows into the cell, the CL reaction will take place. The sample volume and flow rate are critical; for instance, if sample volume is too small or too large, maximum CL could not be obtained. The highest emission was observed if the injected sample volume was 120 µl. The CL intensity increased with increasing flow rate. However, a flow rate of 3.0 ml min^{-1} for all solutions is recommended because of greater precision and economy in the use of reagents.

Effect of Acidic Medium

The nature and concentration of the acid used in the reaction have very significant influences on the CL emission intensity. Therefore, several acids, such as HCl, H_2SO_4, HNO_3, H_3PO_4 and $H_6P_4O_{13}$, were tested. The highest and most stable emission was observed for ADM-$KMnO_4$-HCOH, and MMC-$KMnO_4$-HCOH systems in HNO_3 medium. In the next step, the influence of nitric acid concentration on relative CL intensity was studied and the results are shown in Fig. 3. As is obvious, the optimum acid concentration was 2.5 M HNO_3 for ADM-$KMnO_4$-HCOH and 1.0 MHNO_3 for MMC-$KMnO_4$-HCOH systems.

Effect of $KMnO_4$ Concentration

The concentration of $KMnO_4$ as oxidant was found to

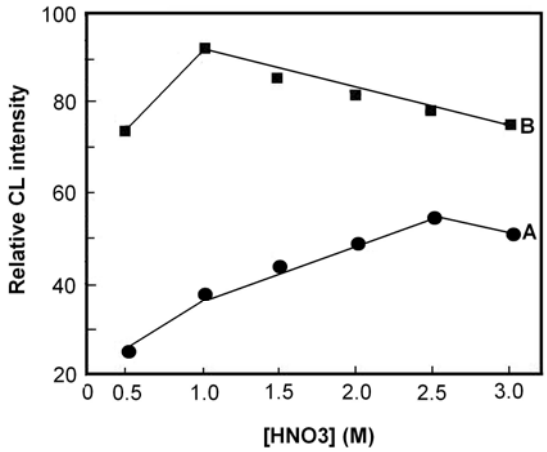

Fig. 3. Effect of nitric acid concentration on relative CL intensity: (A) $KMnO_4 = 5 \times 10^{-4}$ M, HCOH = 2%, ADM = 8×10^{-6} g ml^{-1}; (B) $KMnO_4 = 5 \times 10^{-4}$ M, HCOH = 2%, MMC = 4×10^{-6} g ml^{-1}.

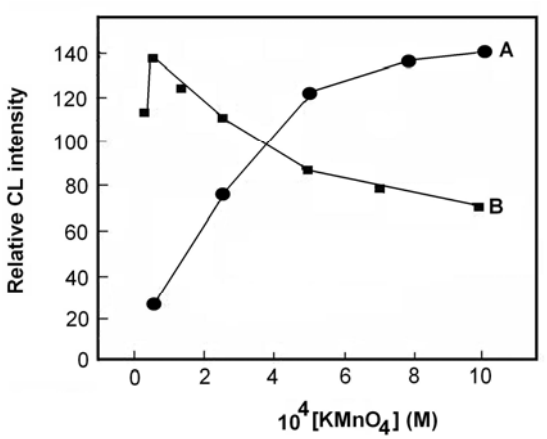

Fig. 4. Effect of potassium permanganate concentration on Relative CL intensity: (A) ADM = 8×10^{-6} g ml^{-1}, HNO_3 = 2.5 M, HCOH = 2.0%; (B) MMC = 4×10^{-6} g ml^{-1}, HNO_3 = 1.0 M, HCOH = 2.0%.

influence not only the sensitivity, but also the linear range of CL system. Therefore, the effect of the $KMnO_4$ concentration on the CL intensity was investigated for 8.0×10^{-6} g ml^{-1} ADM and 4.0×10^{-6} g ml^{-1} MMC and the results are shown in Fig. 4. As can be seen, maximum CL intensity was obtained at $KMnO_4$ concentrations of 5.0×10^{-4} M and 5.0×10^{-5} M for

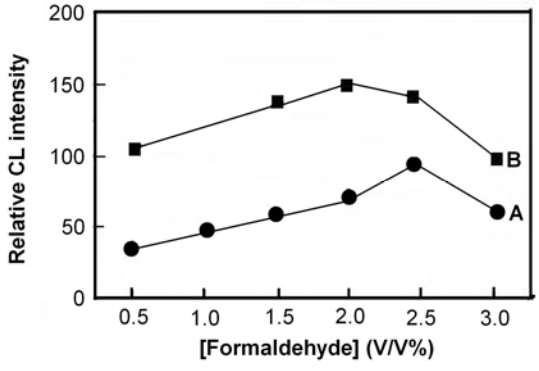

Fig. 5. Effect of formaldehyde concentration on relative CL intensity: (A) ADM = 8×10^{-6} g ml^{-1}, $KMnO_4 = 5 \times 10^{-4}$ M, HNO_3 = 2.5 M; (B) MMC = 4×10^{-6} g ml^{-1}, $KMnO_4 = 5 \times 10^{-5}$ M, HNO_3 = 1.0 M.

ADM and MMC, respectively.

Effect of Sensitizers

In the absence of a sensitizer, both the $KMnO_4$-ADM and $KMnO_4$-MMC systems could only produce weak CL emissions. Thus, various compounds such as rhodamine B, HCOH, H_2O_2, Na_2SO_3, $Na_2S_2O_3$, $Na_2S_2O_4$, were tested as potentioal sensitizers. It was found that, among the copounds tested, only HCOH enhanced the CL signal for the $KMnO_4$-ADM and $KMnO_4$-MMC systems. The effect of HCOH concentration (in the range of 0.5%-3%) on the CL intensity was then investigated and the results are shown in Fig. 5. As is obvious, the CL intensity is enhanced with increasing HCOH concentration up to 2.5% for the $KMnO_4$-ADM system and 2.0% for $KMnO_4$-MMC system and then decreased upon further addition of the acid. Hence, the HCOH working concentration was fixed at 2.5% and 2.0%, for $KMnO_4$-ADM and $KMnO_4$-MMC, respectively.

Kinetic Characteristics of CL Reaction

CL kinetic characteristics of the reactions of ADM-$KMnO_4$-HCOH and MMC-$KMnO_4$-HCOH systems were investigated in detail. It was found that the reaction in acidic medium is very fast. For ADM-$KMnO_4$-HCOH-HNO_3 system, the time needed to reach maximum peak, after reagent

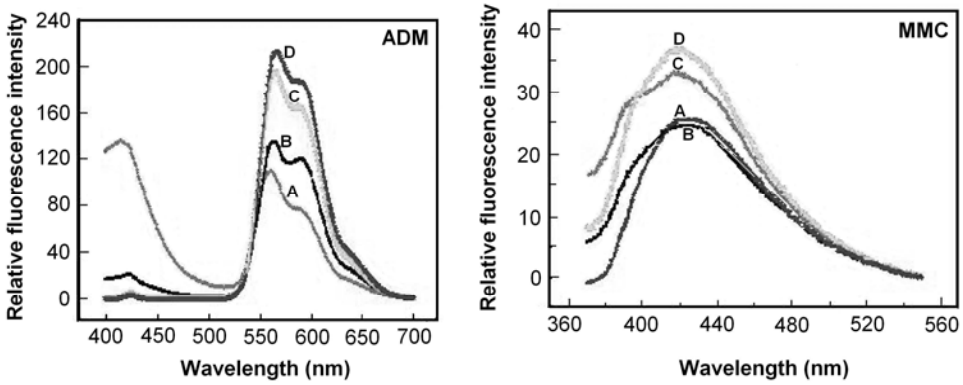

Fig. 6. Fluorescence emission spectra of different systems: (1) ADM-HNO_3 (A), $KMnO_4$-ADM-HNO_3 (B), $KMnO_4$-ADM-HCOH-HNO_3 (C) and ADM-HCOH-HNO_3 (D). Conditions: λ_{ex} = 368 nm, ADM = 2×10^{-4} g ml^{-1}, $KMnO_4$ = 5×10^{-4} M, HCOH = 2.5%, HNO_3 = 2.5 M; (2) MMC-HNO_3 (A), $KMnO_4$-MMC-HNO_3 (B), $KMnO_4$-MMC-HCOH-HNO_3 (C) and MMC-HCOH-HNO_3 (D). Conditions: λex = 293 nm, MMC = 4×10^{-5} g ml^{-1}, $KMnO_4$ = 5×10^{-5} M, HCOH = 2.0%, HNO_3 = 1.0 M.

Table 1. Fluorescence Wavelengths of Different Reaction Systems

Reaction system	Fluorescence wavelength (nm)
ADM-HNO_3	560
ADM-HCOH-HNO_3	566
ADM-$KMnO_4$-HNO_3	417, 566
ADM-$KMnO_4$-HCOH-HNO_3	417, 566
MMC-HNO_3	420
MMC-HCOH-HNO_3	420
MMC- $KMnO_4$-HNO_3	420
MMC-$KMnO_4$-HCOH-HNO_3	420

mixing, and that for the decay of the signal to base line were, respectively, 2.5 s and 8 s for ADM-$KMnO_4$-HCOH-HNO_3, and 3.5 s and 9 s for MMC-$KMnO_4$-HCOH-HNO_3.

Fluorescence Characteristics

The fluorescence spectra for the two drug system were examined in order to obtain more information about their enhanced CL mechanism (Fig. 6) and the fluorescence wavelengths are listed in Table 1.

As can be seen from the spectra shown in Fig. 6A, the native fluorescence emissions of ADM-HNO_3 system in the absence and the presence of HCOH show a broad peak at 560 and 566 nm, respectively, while the fluorescence emissions of this system in the presence of $KMnO_4$ shows two broad peaks at 566 and 417 nm. On the other hand, the native fluorescence emissions of MMC-HNO_3 system in the absence and the presence of HCOH show a broad peak at 420 nm, while the fluorescence emissions of this system with and without $KMnO_4$ always show a broad peak at 420 nm. From Fig. 6 and Table 1 it is indicated that the presence of HCOH could lightly change fluorescence emissions of ADM-HNO_3, and could not change fluorescence emission of MMC-HNO_3 systems. In addition, the presence of $KMnO_4$ couldn't change fluorescence emissions of MMC-HNO_3 systems, but could produce a new

fluorescence peak at 417 nm for ADM-HNO_3, it may be the fluorescence peak of the oxide of ADM [33].

Mechanism of the CL Reaction

The spectroscopic studies have demonstrated that the C2-C3 double bond of indole ring in melatonin and some of its derivatives is oxidized by $KMnO_4$ in the presence of formaldehyde in H_2SO_4 medium, with maximum CL peak being located at 640 nm [26]. For $KMnO_4$-HCOH system with uric acid or methotrexatum as reducing agent, the CL spectra showed a maximum wavelength at 630 nm [34,35]. In an acidic medium, MnO_4^- is reduced to Mn^{2+} and excess of Mn^{2+} could generate Mn^{3+} [36]. Because excess formaldehyde could stabilize manganese as Mn^{2+}, the amount of Mn^{3+} in the solution decreased, resulting in diminution of CL emission [32]. In this work, we obtained very similar results, based on which it could be suggested that HCOH does not change the emitter of the CL reaction, but instead, increases the luminescence quantum number. It may be suggested that a proper HCOH concentration increases the number of singlet oxygens in the CL reaction system.

In order to get an idea about the reaction product of the CL reaction, the CL spectrum of the reaction was examined by a series of interference filters, in the range of 360-730 nm (Fig. 7). It should be noted that, in the absence of ADM and MMC, the CL spectra of $KMnO_4$-HCOH-HNO_3 system showed no obvious CL spectrum.

The fluorescence spectra shown in Fig. 6 revealed that formaldehyde only enhances the fluorescence emission intensity and does not influence the spectra. As shown in Fig. 7, the CL spectrum shows a peak at 649 nm for ADM-$KMnO_4$-HCOH-HNO_3 system and one at 639 nm for MMC-$KMnO_4$-HCOH-HNO_3. The CL peak wavelengths are very different from those reported for the reaction systems listed in Table 1. Singlet state bi-molecule oxygen $^1O_2{}^1O_2$ ($^1\triangle_g{}^1\triangle_g$) is an illuminant and its theoretical maximum wavelength for CL emission is 645 nm [37]. The CL spectrum for the two studied reaction systems shows a peak at 649 or 639 nm, which basically coincided to the theoretical maximum wavelength. The observed deviations are possibly caused by different media.

Thus, the above mentioned observations most possibly insisted the presence of $^1O_2$ ($^1\triangle_g$) (singlet state oxygen) in the reaction of potassium permanganate and organic compounds in acidic media. The presence of HCOH may accelerate the generation of $^1O_2$ ($^1\triangle_g$), which is further transferred to illuminant $^1O_2{}^1O_2$ ($^1\triangle_g{}^1\triangle_g$). When it was transferred to triplet state oxygen $^3O_2$ ($^3\sum g$), the CL spectrum for the two studied reaction systems shows a peak at 649 or 639 nm. Therefore, the mechanism may be summarized as following:

$$MnO_4^- + H^+ + A \rightarrow {}^1O_2({}^1\triangle_g) + Mn^{2+} + A^\bullet + H_2O$$

$$2MnO_4^- + 6H^+ + 5CHOH + 2H_2O \rightarrow 5HOCH_2OOH + 2Mn^{2+}$$

$$2HOCH_2OOH + A^\bullet \rightarrow HCHO + HCOOH + {}^1O_2({}^1\triangle_g) + A + H_2O$$

$${}^1O_2({}^1\triangle_g) + {}^1O_2({}^1\triangle_g) \rightarrow {}^1O_2{}^1O_2({}^1\triangle_g{}^1\triangle_g)$$

$${}^1O_2{}^1O_2({}^1\triangle_g{}^1\triangle_g) \rightarrow {}^3O_2({}^3\Sigma_g) + h\nu$$

where A is the analyte (ADM, MMC) and $A^\bullet$ is the radical products.

Interference Studies

The influence of some common excipients used in drugs, metal ions present in human body and several different organic compounds on the CL intensity of a 4×10^{-6} g ml^{-1} solution of analyte was investigated by comparing the CL emission obtained from an ADM solution alone with that for analyte in the presence of the foreign species added. The tolerance limit was defined as the amount of foreign species that produced an error not exceeding ±5%. The resulting tolerated concentration ratios (interference/analyte) are given in Table 2. Besides the data given in Table 2, there was feeble interferences from equal amounts of hemoglobin, myoglobin and vitamin B_1.

Analytical Performance of CL System

Under the optimum conditions described above, the linearity and relative standard deviation (RSD) for detection of ADM and MMC were investigated by using the proposed system. The calibration graphs of ADM and MMC were consisted of three or four parts in order to improve the veracity. Regression equations are listed in Table 3, along with the detection limits and relative standard deviations.

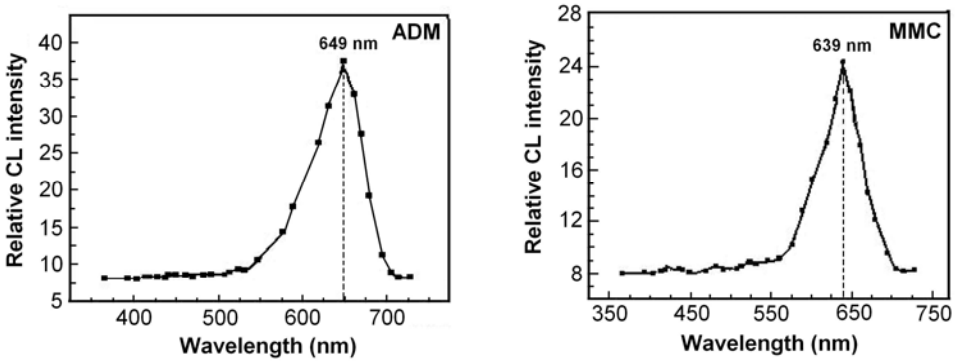

Fig. 7. CL spectra of different reaction systems: (1) ADM-$KMnO_4$-HCOH-HNO_3 system. $KMnO_4 = 5 \times 10^{-4}$ M, HCOH= 2.5%, $HNO_3 = 2.5$ M, ADM= 2×10^{-4} g ml^{-1}; (2) MMC-$KMnO_4$-HCOH-HNO_3 system. $KMnO_4 = 5 \times 10^{-5}$ M, HCOH = 2.0%, $HNO_3 = 1.0$ M, MMC = 1×10^{-5} g ml^{-1}.

Table 2. Tolerance Concentration Ratio of Foreign Species for ADM and MMC at 4×10^{-6} g ml^{-1}

Foreign species	Tolerance concentration ratio	
	ADM	MMC
Magnesium stearate	100	100
Fructose, Dextrin, Galactose	100	100
Sucrose, Sodium citrate	100	100
Lactose	25	50
Starch	100	25
Glucose		25
Sodium benzoate		100
Zn^{2+}, Mg^{2+}, Co^{2+}, Ba^{2+}, Al^{3+}	100	100
Ni^{2+}, Ca^{2+}, SO_4^{2-}, Cl^-, NO_3^-	100	100
Cu^{2+}	25	
Fe^{3+}	10	100
Br^-	10	10
I^-	2	
NO_2^-		5
Polyglycol	2	

The limit of detection was determined as the sample concentration that produces a peak with a height three times of the level of baseline noise [38,39]. The detection limits (3σ) for the first equations of ADM and MMC were 3×10^{-8} and 3×10^{-9} g ml^{-1}, respectively. The relative standard deviations were found to be 2.2% and 1.8% for 11 determinations of 2.0 $\times 10^{-6}$ g ml^{-1} of ADM and 2.0×10^{-7} g ml^{-1} of MMC, respectively. The results thus obtained indicate that the proposed CL systems possess good linearity, high sensitivity and precision.

Pharmaceutical Analysis

In order to evaluate the validity of the proposed method, the recovery of ADM and MMC from pharmaceuticals was investigated and the results are given in Table 4. As seen, the recoveries are in the range of 95.0-105.0%.

The proposed method was also applied to the determination of ADM and MMC in some injection samples and the results are summarized in Table 5, along with the labeled contents. It should be noted that the results agree well with those obtained by a standard method [40]. There was also no significant difference between the labeled contents and the results obtained by the proposed method. The reproducibility of the proposed method was calculated in terms of the variation coefficient from the CL intensity of seven independent replicate analyses, which resulted in a relative standard deviation of 2.1%.

CONCLUSIONS

A novel CL reaction system was established for the assay of the anticancer drugs adriamycin (ADM) and mitomycin (MMC). The CL spectra for ADM-$KMnO_4$-HCOH-HNO_3 or MMC-$KMnO_4$-HNO_3 system, showed a peak at 649 or 639 nm,

Table 3. Regression Equation, Detection Limit and RSD for Determinations of the Two Anticancer Drugs

Analyte	Regression equation	Correlation coefficient	Linear range ($g\ ml^{-1}$)	Detection limit ($g\ ml^{-1}$)	RSD (%)[a]
ADM	$I = 1.0C + 49.7$	0.9989	1.0×10^{-7}-1.0×10^{-6}	3×10^{-8}	2.2
	$I = 4.0C + 54.1$	0.9979	1.0×10^{-6}-1.0×10^{-5}		
	$I = 19.0C + 77.6$	0.9981	1.0×10^{-5}-1.0×10^{-4}		
MMC	$I = 1.1C + 17.7$	0.9989	1.0×10^{-8}-1.0×10^{-7}	3×10^{-9}	1.8
	$I = 3.6C + 25.0$	0.9988	1.0×10^{-7}-1.0×10^{-6}		
	$I = 25.6C + 44.8$	0.9963	1.0×10^{-6}-1.0×10^{-5}		
	$I = 24.9C + 267.1$	0.9988	1.0×10^{-5}-6.0×10^{-5}		

[a]for 11 determinations of ADM at 2.0×10^{-6} g ml^{-1} and MMC at 2.0×10^{-7} g ml^{-1}.

Table 4. Recovery Experiments

Analyte	Content (10^{-6} g ml^{-1})	Added (10^{-6} g ml^{-1})	Found (10^{-6} g ml^{-1})	Recovery (%)	RSD (%) n = 7
ADM	0.29	0.2	0.48	95.0	2.3
		0.4	0.70	102.5	2.0
		0.6	0.88	98.3	2.1
	2.55	2.0	4.56	100.5	1.9
		4.0	6.54	99.8	2.0
		6.0	8.65	101.7	1.8
MMC	0.19	0.2	0.40	105.0	2.0
		0.4	0.58	97.5	1.8
		0.6	0.81	103.3	1.9
	2.35	2.0	4.34	99.5	1.7
		4.0	6.40	101.3	1.6
		6.0	8.53	103.0	1.9

Table 5. Determination Results of ADM in Sample

Analyte	Injection batch number	Nominal (mg/bottle)	Proposed method (mg/bottle)	RSD (%) n = 7	UV method (mg/bottle)
ADM[a]	021101	10	10.07	2.1	10.10
	030201	10	10.12	2.0	10.13
	011210	10	10.03	2.1	10.05
MMC[b]	03021621	2	1.98	2.1	2.01
	04061520	2	2.01	2.0	2.02

[a]Haizheng Pharmaceuticals Ltd., Co. [b]Hengrui Pharmaceuticals Ltd., Co.

respectively, which coincided basically to a reported theoretically maximum wavelength. A singlet state bi-molecule oxygen $O_2{}^1O_2$ ($^1\triangle_g{}^1\triangle_g$) was suggested to be illuminant. Under optimum conditions, the proposed CL systems possess good linearity, high sensitivity and precision and revealed potential capability for the analysis of these drugs in biological samples and investigation of their anticancer activity.

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