Analytical Methods

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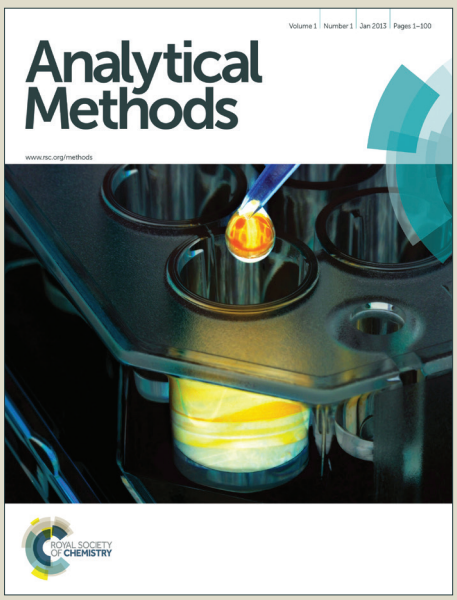

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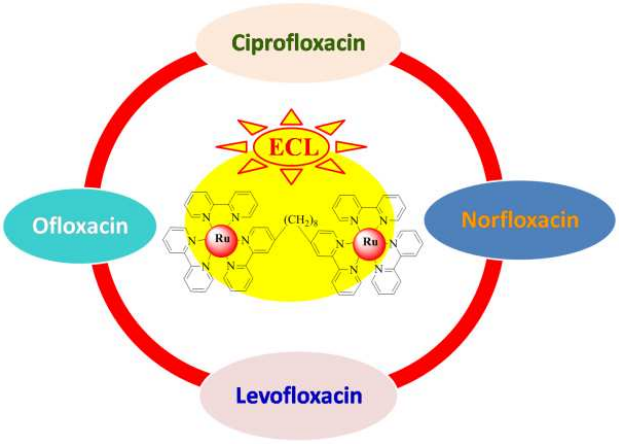
Graph abstract



Electrochemiluminescence (ECL) of bimetallic ruthenium *tris*-bipyridyl complex $[(bpy)_2Ru(bpy)(CH_2)_8(bpy)Ru(bpy)_2]^{4+}$ has been employed for the determination of ofloxacin (OFLX), levofloxacin (LVFX), norfloxacin (NFLX) and ciprofloxacin (CPFX) in milk samples with good reproducibility and stability.

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ARTICLE

Detection of Quinolone Antibiotics with Electrochemiluminescence of Bimetallic Ruthenium Complex $[(bpy)_2Ru(bpy)(CH_2)_8(bpy)Ru(bpy)_2]^{4+}$

Fengyu Liu,*[a] Yulong Gao,[a] Yinqi Zhao[b]

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Electrochemiluminescence (ECL) of bimetallic ruthenium *tris*-bipyridyl complex $[(bpy)_2Ru(bpy)(CH_2)_8(bpy)Ru(bpy)_2]^{4+}$ at a glassy carbon electrode has been employed for the determination of the four widely used quinolone antibiotics. The method gave a linear response from 1.0×10^{-13} to 1.0×10^{-6} mol/L, 1.0×10^{-14} to 1.0×10^{-7} mol/L, 1.0×10^{-15} to 1.0×10^{-6} mol/L, and 1.0×10^{-15} to 1.0×10^{-6} mol/L concentration ranges for ofloxacin, levofloxacin, norfloxacin, and ciprofloxacin, respectively. Remarkable quantitation limits of 1.0×10^{-13}, 1.0×10^{-14}, 1.0×10^{-15}, and 1.0×10^{-15} mol/L were observed for the detection of ofloxacin, levofloxacin, norfloxacin, and ciprofloxacin in phosphate buffer (0.10 mol/L). These detection levels were much lower than those observed for other detection methods. The proposed method was successfully employed in the determination of ofloxacin, levofloxacin, norfloxacin, and ciprofloxacin after their addition into milk samples. Good reproducibility and stability were observed. This method allows for the development of an ECL-based method for the detection of quinolone antibiotic residues.

Introduction

Quinolones, an important family of synthetic antibacterial compounds, have been widely used against bacterial infections in both humans and animals. Unfortunately, this can cause allergic reactions in sensitive individuals. Along with the extensive use of quinolone antibiotics in animal husbandry and aquaculture, the side effects and adverse effects of them get more and more attention[1]. Furthermore, the ingestion of sub-therapeutic doses of antibiotics may lead to the development of drug-resistant strains of bacteria. The European Union (EU) and the joint FAO/WHO Expert committee on Food additives (JECFA) have set maximum residue limits (MRLs) for several quinolones in foodstuffs of animal origin (directive 2377/90/EEC)[2]. The MRL for norfloxacin in edible tissues of poultry/pigs is 50 μg/kg and the same for ofloxacin in milk is 75 μg/kg. Methods such as high performance liquid chromatography (HPLC)[3-8], liquid chromatography coupled with mass spectrometry (LC-MS)[9,10], spectrophotometry[11], fluorescence spectrophotometry[12,13] and chemiluminescence[14-16] have been utilized to measure quinolone antibiotics. Most of these methods are time-consuming and their execution requires special training. Development of a simple and reliable method for rapid and sensitive determination of quinolone antibiotics remains a challenge.

Deng and coworkers[17] have developed a fast and sensitive approach to detect norfloxacin in human urine using capillary electrophoresis with end-column $Ru(bpy)_3^{2+}$ electrochemiluminescence (ECL). The ECL intensity varied linearly with norfloxacin concentration in the 5.0×10^{-8}–1.0×10^{-5} mol/L range. The detection limit (with a signal-to-noise ration S/N=3) was 4.8×10^{-9} mol/L. The application of this method was extended to the determination of quinolones in pig urine after clean-up by C_{18} solid phase extraction[18]. The detection limits (S/N=3) for enrofloxacin, levofloxacin, and ciprofloxacin were 2.8×10^{-8} mol/L, 2.8×10^{-8} mol/L, and 2.1×10^{-8} mol/L, respectively. In an alternate method, 7-Piperazinyl fluoroquinolone antibiotics were employed as a co-reactant with $Ru(bpy)_3^{2+}$ as the ECL reagent in a flow injection analysis (FIA) system[19].The method linear range, precision, detection limits, and sensitivity for the detection of enrofloxacin and ciprofloxacin were comparable to that of tripropylamine (TPrA), the most widely used amine reductant in ECL. The $Ru(bpy)_3^{2+}$/TPrA system has been studied extensively and forms the basis of commercial ECL systems[20].

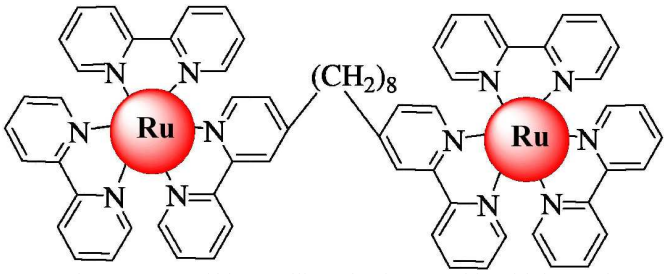

Fig. 1 The structure of biometallic ruthenium *tris*-bipyridyl complex (**1**)

In our previous work[21], the quantitation limit for TPrA was lowered to 0.1 fM at glassy carbon (GC) electrode with the use of a bimetallic ruthenium *tris*-bipyridyl complex $[(bpy)_2Ru(bpy)(CH_2)_8(bpy)Ru(bpy)_2]^{4+}$ (**1**, Fig. 1) as the ECL

label. Very sensitive ECL response was observed on Au electrode, while the amount TPrA needed was only one-fifth of that required in the $Ru(bpy)_3^{2+}$/TPrA system. To extend the application of the bimetallic ruthenium complex, **1**, was employed in the detection of the four widely used quinolone antibiotics, ofloxacin (OFLX), levofloxacin (LVFX), norfloxacin (NFLX), and ciprofloxacin (CPFX) (Fig. 2). The results demonstrate that both a wider linear response concentration range, and a remarkably lower quantitation limit can be achieved; this methods can detect levels of quinolones below the MRLs set by the European Union (EU) and the joint FAO/WHO Expert Committee on Food Additives (JECFA).

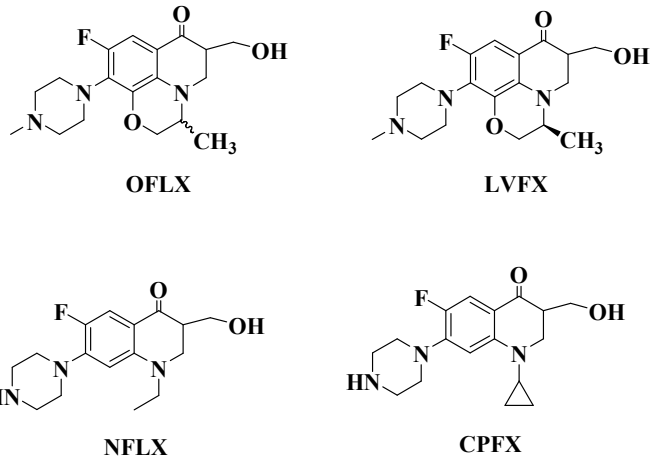

Fig. 2 The structures of the four quinolone antibiotics.

Experimental

Chemicals and reagents

PF_6^- (hexafluorophosphate) salts of **1** was synthesized in our lab[21], Standard samples of ofloxacin (OFLX, 99.0%), levofloxacin (LVFX, 99.0%), norfloxacin (NFLX, 99.0%) , ciprofloxacin hydrochloride (CPFX, 99.0%) were purchased from State Food and Drug Administration, P. R. China. Other chemicals and solvents were all of reagent grade and used as received. Milks were commercial samples obtained from a local supermarket.

Preparation of standard solutions and milk samples

The 1.0×10^{-3} mol/L standard stock solutions of OFLX, LVFX, and NFLX were prepared by accurately weighting 18.1 mg of OFLX, 18.1 mg of LVFX, and 16 mg of NFLX, added into three 50 mL volumetric flask and dissolved in acetonitrile, respectively. Then, the stock solutions of OFLX, NFLX and LVFX were diluted respectively with acetonitrile to obtain nine standard solutions, 1.0×10^{-12}, 1.0×10^{-11}, 1.0×10^{-10}, 1.0×10^{-9}, 1.0×10^{-8}, 1.0×10^{-7}, 1.0×10^{-6}, 1.0×10^{-5}, and 1.0×10^{-4} mol/L respectively. The 1.0×10^{-3} mol/L standard stock solution of CPFX was prepared by accurately weighting 18.4 mg of ciprofloxacin hydrochloride, added into 50 mL volumetric flask and dissolved in deionized water. The stock solution of CPFX was diluted with deionized water to obtain nine standard solutions, 1.0×10^{-12}, 1.0×10^{-11}, 1.0×10^{-10}, 1.0×10^{-9}, 1.0×10^{-8}, 1.0×10^{-7}, 1.0×10^{-6}, 1.0×10^{-5}, and 1.0×10^{-4} mol/L respectively. All the solutions were kept at room temperature.

The milk samples were prepared according to modified method of the literature[22,23]. In short, 4 ml 20% (4.2×10^{-3} mol/L) trichloroacetic acid was added into 20 ml commercial milk and shaken for 10 min to make protein coagulation. 10 ml sample-extracting solvent (the volume ration of ethanol and acetonitrile is 1:1) was added and vortexed for 10 min. Then the mixture was centrifuged at 16,000 rpm for 10 min and heat-treated 3 min at 70 ºC, to inactivate endogenous substances. The supernatant was transferred to a 50 ml volumetric flask. The residue was further extracted with 16 mL sample-extracting solvent (8 mL × 2) and the extract was collected and combined with the supernatant. Then the corresponding OFLX, NFLX, LVFX and CPFX sample was added into the volumetric flask. The volumetric flask was filled to the final volume with acetonitrile. In this way, the interfering substances in the milk such as high concentrations of calcium and protein etc. can be removed. The milk samples containing different concentration of quinolone antibiotics were acquired.

ECL measurement

ECL measurements were performed with a MPI-B multifunctional ECL detector (Xi'an Remax Electronics, Xi'an, China). All experiments were carried out at room temperature. A commercial cylindroid quartz cell was used as an ECL cell, which contained a conventional three-electrode system consisting of GC, Au and Pt as the working electrode, a KCl-saturated Ag/AgCl electrode and a platinum wire electrode were used as the reference and the auxiliary electrode, respectively. The working electrodes were polished and cleaned ultrasonically in sulfuric acid, nitric acid and deionized water, wiped dry before measurement.

The corresponding standard solution (5.0 μL) of quinolone antibiotics or milk sample containing quinolone antibiotics was added into 5.0 mL 1.0×10^{-4} mol/L **1** in phosphate buffer. The mixture was transferred to an ECL detection cell for ECL determination. Cyclic potential sweep experiments were carried out in the potential region from 0.2 to 2.0 V, and then back to 0.2 V at a scan rate of 100 mV/s, the ECL signals and CV vs time were measured rspeatedly for at least 7 times, and the averaged readings were used for the creation of plots.

Experimental precision determination

The intra-day precision was determined by employing 1.0×10^{-4} mol/L **1** to analyze the standard milk samples containing 1.0×10^{-9} mol/L quinolone antibiotics for six times on the same day, while inter-day precision was determined by employing 1.0×10^{-4} mol/L **1** to analyze the standard milk samples containing 1.0×10^{-9} mol/L quinolone antibiotics daily for 6 days over a period of one week.

Results and discussion

Optimization of pH

Phosphate buffer (0.10 mol/L) was employed, and the initial pH of the solution was adjusted with concentrated NaH_2PO_4 and Na_2HPO_4 solution to the required pH value. The pH of the buffer solution has been reported to play an important role in aqueous ECL reactions[24], therefore, the ECL performance of **1** (1.0×10^{-5} mol/L) in the presence of quinolone antibiotics (1.0×10^{-6} mol/L) was evaluated under different pHs. As illustrated in Fig. 3, both the highest ECL signal and the highest signal-to-background noise ratio were observed at pH values of 7.0 for NFLX (The pH optimization result of NFLX is included here as an example; other results are shown in Fig. S1). Multiple replicate measurements with relative standard deviations (RSDs) that were < 5.0% suggested good stability and reproducibility of the analytical method under these conditions. Subsequently, solutions of phosphate buffer (0.10 mol/L) with pH values at

7.0 were used in the ECL measurement of NFLX. For the same reason, phosphate buffer (0.10 mol/L) with pH values at 6.5, 6.0 and 7.0 was used in the ECL measurement of OFLX, LVFX and CPFX, respectively.

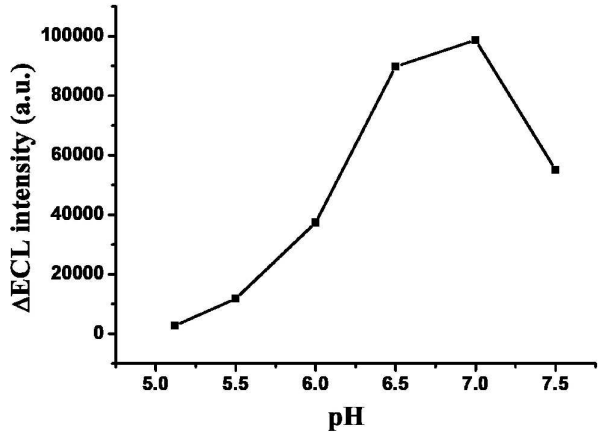

Fig. 3 Dependence of the ECL increase versus the pH with 1.0×10^{-5} mol/L **1** and 1.0×10^{-6} mol/L NFLX in 0.10 mol/L phosphate buffer at GC electrode. $\Delta ECL = ECL_{after\ addition\ of\ analyte} - ECL_{before\ addition\ of\ analyte}$.

Optimization of scan rate

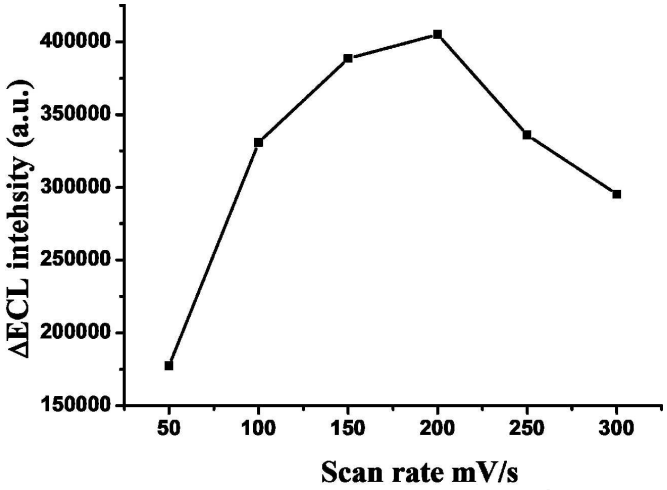

Fig. 4 The effect of different scan rate on ECL with 1.0×10^{-5} mol/L **1** and 1.0×10^{-6} mol/L NFLX in 0.10 mol/L phosphate buffer (pH=7.0 at GC electrode. $\Delta ECL = ECL_{after\ addition\ of\ analyte} - ECL_{before\ addition\ of\ analyte}$.

As indicated in the literature[25], the scan rate affected the ECL over a wide range, because the ECL efficiency was significantly dependent on the rate of generation/annihilation of the excited state $*Ru(bpy)_3^{2+}$ [26]. ECL performance of **1** (1.0×10^{-5} mol/L) and corresponding quinolone antibiotics (1.0×10^{-6} mol/L) in phosphate buffer (0.10 mol/L; at solution pH values of 6.5, 6.0, 7.0, and 7.0 for OFLX, LVFX, NFLX, and CPFX, respectively) at GC electrode under different scan rates were also evaluated. It is evident from Fig.4 (The scan rate optimization result of NFLX is included here as an example; other results are shown in Fig.S2) that the scan rate significantly affected the ECL at GC electrode in phosphate buffer (0.10 mol/L). Although the highest ECL can be achieved at 250 mV/s, the best reproducibility and stability were shown under the condition of 100 mV/s, so 100 mV/s was employed for all the detection of NFLX below. For the same reason, 100 mV/s was also employed for the detection of the other quinolone antibiotics below.

ECL performance of 1 in the presence of quinolone antibiotics

In order to examine the ECL response of **1** to quinolone antibiotics, **1** (1.0×10^{-4} mol/L) was titrated with different concentrations of quinolone antibiotics in phosphate buffer (0.10 mol/L). When the electrode potential was scanned in the positive direction (~1.0 V), ECL signal was observed upon the oxidation of **1**, in accordance with the literature[21,27,28]. Significant enhancement in ECL was observed when the electrode potential was ~1.1 V. The ECL intensity of **1** at GC electrode increased with increase in the concentration of quinolone antibiotics in phosphate buffer.

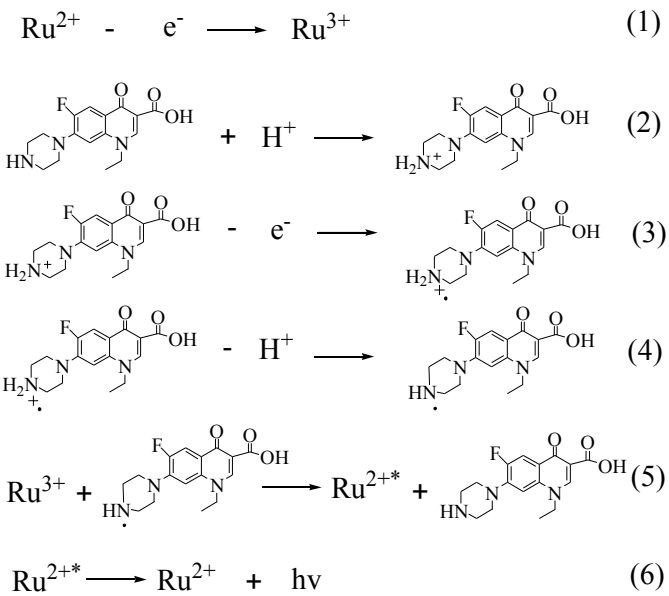

Fig. 5 The proposed mechanism for **1** or $Ru(bpy)_3^{2+}$/ quinolone antibiotics ECL system (Ru^{2+} stands for $Ru(bpy)_3^{2+}$ or **1**)

Based on previous reports[29-31], a mechanism for the observed ECL was proposed (Fig.5; where NFLX represented all the quinolones). NFLX first underwent a one-electron oxidation in either step 2 and 3 and formed the NFLX cation radical ($^{+\cdot}$NFLX), which was rapidly deprotonated to form an NFLX free radical ($^{\cdot}$NFLX). This highly energetic NFLX radical generated the excited state species Ru^{2+*} through reduction of Ru^{3+} in step 5 (Ru^{2+} represented $Ru(bpy)_3^{2+}$ or **1**).

A good linear fit for the calibration curve (Fig. 6) for ΔECL ($\Delta ECL = ECL_{after\ addition\ of\ NFLX} - ECL_{before\ addition\ of\ NFLX}$) with the logarithmic concentration of NFLX (log[NFLX]) was observed over 1.0×10^{-15}–1.0×10^{-6} mol/L NFLX. The regression equation was $\Delta ECL = 91583.57 + 5541.97 \times \log[NFLX]$ with a linear coefficient $R^2 = 0.99$. The quantitation limit for NFLX was 1.0×10^{-15} mol/L (3.19×10^{-13} g/L), which is much lower than the MRLs (the MRLs of NFLX in edible tissues of poultry/pigs and milk are 50 μg/kg and 100 μg/kg, respectively) set by the European Union (EU) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The calibration curve of NFLX is included here as an example; other calibration curves are shown in Figs. S3–S5 (supporting information).

For the experiments that determined the concentration of NFLX using the commercially available metallic $Ru(bpy)_3^{2+}$, the linear range was from 1.0×10^{-11} to 1.0×10^{-6} mol/L and

the quantitation limit was 1.0×10^{-11} mol/L, which is at least 4 orders of magnitude higher than that for the determination of NFLX with **1**. This demonstrated the superiority of **1** in detecting quinolone antibiotics at GC electrode. A similar trend was observed when Au and Pt electrodes were used (Fig. S6); similar detection ranges (1.0×10^{-13}–1.0×10^{-6} mol/L) and quantitation limits (~1.0×10^{-13} mol/L) were observed, indicating the generality of the method with different electrode.

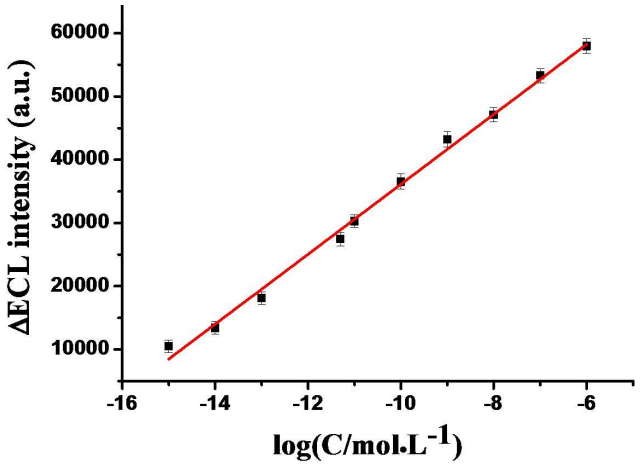

Fig. 6 Dependence of the ΔECL increase versus the logarithmic of NFLX with 1.0×10^{-4} mol/L **1** in 0.10 mol/L phosphate buffer (pH=7.0) at GC electrode. $\Delta ECL = ECL_{\text{after addition of NFLX}} - ECL_{\text{before addition of NFLX}}$

Compared with NFLX and CPFX, higher ECL intensities were observed with OFLX and LVFX. This can be ascribed to the presence of the tertiary nitrogen in the piperazine groups of OFLX and LVFX (only a secondary nitrogen is present in NFLX and CPFX). These observations were in good agreement with the results reported in literature[19,32].

The wide linear response concentration range and the remarkable quantitation limit were clearly associated with the soft carbon chain which linked the two ruthenium moieties intramolecularly without imposing significant steric hindrance on each ruthenium active center either at the electrode surface or in the solution[21]. Meanwhile, both the ruthenium centers could simultaneously approach the target quinolone antibiotic, leading to a combination of oxidation-reduction[20] and self-annihilation[22] types of ECL. The two ruthenium moieties in **1** could work like a 'pincer' to embrace the quinolone antibiotic tightly; this is illustrated by the geometry-optimized structures (obtained using HyperChem v. 8.0) shown in Fig. S7.

Considering that two ruthenium active centers existed in **1**, the ECL performance of **1** (5.0×10^{-5} mol/L) was compared with the reference complex, $Ru(bpy)_3^{2+}$ (1.0×10^{-4} mol/L) that contained a single active site, and the obtained linear relationships are presented in Figs. S8–S11. Although the concentration of **1** is half that of $Ru(bpy)_3^{2+}$ (i.e., the same concentration of active metal centers), the ECL intensity due to **1** was significantly higher than that due to $Ru(bpy)_3^{2+}$ in the presence of either of the four quinolone antibiotics. The linear range for the detection of quinolones in presence of **1** was two orders of magnitude wider, and the quantitation limit was two orders of magnitude lower than that with $Ru(bpy)_3^{2+}$.

Interference study

To evaluate the ability of the proposed method in the analysis of quinolone antibiotics in real samples, the effects of potential interference due to the presence of other chemicals in complex sample matrices were also examined. The solutions used for this purpose independently contained 1.0×10^{-13} mol/L, 1.0×10^{-9} mol/L, and 1.0×10^{-6} mol/L of each quinolone antibiotics together with **1** (1.0×10^{-4} mol/L) and the interfering species. A species was considered not to interfere if it caused a relative error of less than ±5.0% in the measurement of 1.0×10^{-13} mol/L, 1.0×10^{-9} mol/L, and 1.0×10^{-6} mol/L of the quinolone antibiotics[9,14].

Concentrations of the interfering compounds relative to 1.0×10^{-6} mol/L quinolone antibiotics tolerated in their ECL detection were determined to be the following: 1,000-fold higher concentration, by weight, for K^+, Na^+, CO_3^{2-}, NO_3^-, Cl^-, sucrose, and fructose; 500-fold higher concentration, by weight, for glucose and glycine; 200-fold higher concentration, by weight, for Mg^{2+}; 100-fold weight concentration for lysine; 50-fold higher concentration, by weight, for arginine; and 10-fold higher concentration, by weight, for Cu^{2+}, Ca^{2+}, aspartic acid, and starch.

The tolerated concentration ratios for detecting 1.0×10^{-9} mol/L quinolone antibiotics were: one million-fold higher concentration, by weight, for K^+, Na^+, CO_3^{2-}, NO_3^-, Cl^-, sucrose, and fructose; five hundred thousand-fold higher concentration, by weight, for glucose; two hundred thousand-fold higher concentration, by weight, for Mg^{2+} and glycine; one hundred thousand-fold higher concentration, by weight, for lysine; 5,000-fold higher concentration, by weight, for arginine; and 10,000-fold higher concentration, by weight, for Cu^{2+}, Ca^{2+}, and starch.

Similarly, the concentration ratios of interfering compounds tolerated in the detection of 1.0×10^{-13} mol/L quinolone antibiotics were: Ten billion-fold higher concentration, by weight, for K^+, Na^+, CO_3^{2-}, NO_3^-, Cl^-, sucrose, and fructose; five billion-fold higher concentration, by weight, for glucose; two billion-fold higher concentration, by weight, for Mg^{2+} and glycine; one hundred million-fold higher concentration, by weight, for Cu^{2+}, Ca^{2+}, and starch; and one hundred thousand-fold higher concentration, by weight, for lysine and arginine. In short, this method was satisfactorily tolerant towards interferences from several chemicals.

Analytical applications

To further assess the accuracy of the proposed method, the quantities of OFLX, LVFX, NFLX, and CPFX were determined after their addition into commercial milk samples. Before the addition of quinolone antibiotics, the milk samples were assessed by LC-MS[9,10] to ensure the absence of quinolone antibiotics. To eliminate interference from high concentrations of calcium and other ingredients, milk samples were prepared as reported previously,[22,23] but with modifications as described in the experimental section. The analytical results in the case of NFLX are shown in Fig. 7 and Table 1 (results for other quinolones can be found in supporting information Figs. S12–S14 and Table S1).

A good linear calibration curve (Fig. 7) between ΔECL ($\Delta ECL = ECL_{\text{after addition of the milk samples}} - ECL_{\text{before addition of the milk samples}}$) and the logarithmic concentration of NFLX (log[NFLX]) was established over the concentration range from 2.6×10^{-14} to 2.6×10^{-6} mol/L. The regression equation was $\Delta ECL = 98908.02 + 6166.15 \times \log[NFLX]$ with a linear coefficient $R^2 = 0.99$. The NFLX quantitation limit was 2.6×10^{-14} mol/L (8.29×10^{-12} g/L), which is much lower than the suggested MRLs.

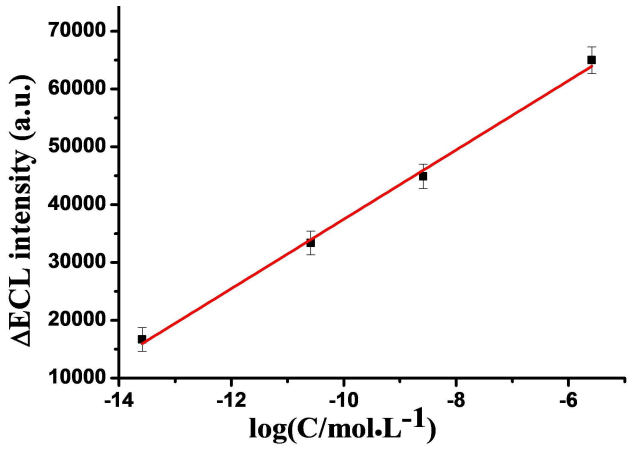

Fig. 7 Dependence of the ΔECL increase versus the logarithmic concentration of the NFLX in milk samples with 1.0×10^{-4} mol/L **1** in 0.10 mol/L phosphate buffer (pH 7.0) at GC electrode. ΔECL = $ECL_{\text{after addition of the milk samples}} - ECL_{\text{before addition of the milk samples}}$

Table 1. Recovery of NFLX added into milk samples detected by ECL of **1** (1.0×10^{-14} mol/L) in pH 7 phosphate buffer (0.10 mol/L).[a]

Added (mol/L)	Detected (mol/L)	Average (mol/L)	Recovery (%)	RSD (%)
2.6×10^{-14}	2.59×10^{-14}			
2.6×10^{-14}	2.60×10^{-14}	2.60×10^{-14}	100.00	0.59
2.6×10^{-14}	2.62×10^{-14}			
2.6×10^{-11}	2.61×10^{-11}			
2.6×10^{-11}	2.59×10^{-11}	2.61×10^{-11}	100.38	0.76
2.6×10^{-11}	2.63×10^{-11}			
2.6×10^{-9}	2.59×10^{-9}			
2.6×10^{-9}	2.61×10^{-9}	2.59×10^{-9}	98.50	0.59
2.6×10^{-9}	2.58×10^{-9}			
2.6×10^{-6}	2.70×10^{-6}			
2.6×10^{-6}	2.60×10^{-6}	2.66×10^{-9}	102.40	2.08
2.6×10^{-6}	2.69×10^{-6}			

[a] Average of three samples, each sample was measured repeatedly for at least 7 times, and the averaged readings were used.

The recovery was quite satisfactory for GC electrode (Table 1). The relative standard deviations of <2.08% and recoveries of 98.50–102.40% for NFLX underscored the accuracy and further demonstrated the potential application of this method for the determination of quinolone antibiotics residues present in milk. Using the described method, it took <90 min (sample preparation included) to determine the quantities of quinolones in a commercial milk sample and the operator had to execute a simple protocol once the parameters of the method (detection conditions, linearity, and calibration curves) had been established. This method used only 0.92 mg of **1** for each determination, underscoring its ability to save time and ECL label.

In order to highlight the advantage of the proposed method, the result of using the proposed method to detect quinolone antibiotics in milk was compared with other analytical methods in table 2. It is noted that the linear range for the detection of quinolones with ECL of **1** was 5-7 orders of magnitude wider, and the quantitation limit was 5-7 orders of magnitude lower than that with other detection methods. All these proved that the proposed method has more advantages for detecting quinolone antibiotics than other detection methods.

Table 2. The comparison of the proposed method and other analytical methods

Analyte	Analytical method	Quantitation range (mol/L)	LOQ [a] (mol/L)
OFLX	The ECL detection method of **1**	2.40×10^{-12} -2.40×10^{-7}	2.40×10^{-12}
	LC-MS method[9,10]	3.04×10^{-7}-7.61×10^{-7}	3.04×10^{-7}
LVFX	The ECL detection method of **1**	1.00×10^{-13} -1.00×10^{-6}	1.00×10^{-13}
	LC-MS method[9,10]	3.04×10^{-7}-7.61×10^{-7}	3.04×10^{-7}
NFLX	The ECL detection method of **1**	2.60×10^{-14} -2.60×10^{-6}	2.60×10^{-14}
	Fluorescence method[12]	1.25×10^{-6} -2.80×10^{-5}	1.25×10^{-6}
CPFX	The ECL detection method of **1**	3.00×10^{-14} -3.00×10^{-6}	3.00×10^{-14}
	HPLC[3-8]	2.47×10^{-7}-8.30×10^{-7}	2.47×10^{-7}

[a] LOQ stands for the limit of quantitation.

Precision studies

In order to assess the experimental precision, **1** (1.0×10^{-4} mol/L) was employed in the detection of quinolone antibiotics in milk samples according to the method mentioned in experimental section. The relative standard deviations for intra- and inter-day variations are given in Tables S2–S5. The low relative standard deviations (<2.50%) for the four quinolone antibiotics established the precision of the method.

Conclusions

In conclusion, ECL of $[(bpy)_2Ru(bpy)(CH_2)_8(bpy)Ru(bpy)_2]^{4+}$ has been successfully employed for the determination of four quinolone antibiotics. The recovery is quite satisfactory and the method shows good reproducibility, precision, and stability. This method allows the development of a sensitive ECL-based detection method for the rapid detection of quinolone residues in milk. The application of this method in the detection of other class of antibiotics is currently being pursued.

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Notes and references

[a] State key laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology, 2 Lingong Road, Ganjingzi District, Dalian 116023, China, Fax:+86 411 84986304, E-mail: liufengyu@dlut.edu.cn
[b] Cangzhou Normal Institute, Cangzhou 061001, Hebei, China.

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