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# Simple, stable and sensitive electrogenerated chemiluminescence detector for high-performance liquid chromatography and its application in direct determination of multiple fluoroquinolone residues in milk

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## ABSTRACT

A simple, stable and sensitive electrogenerated chemiluminescence (ECL) detector was developed. It was based on tris(2,2-bipyridyl)ruthenium(II) ($Ru(bpy)_3{}^{2+}$) immobilized on the surface of a Pt wire with Nepem-105D ion exchange solution. The detector was prepared by inserting a Pt wire with immobilized $Ru(bpy)_3{}^{2+}$ (working electrode) into a capillary tube, followed by inserting another Pt wire (counter electrode) in this tube and sealing. ECL behavior was investigated using ofloxacin as an analyte. Under optimal conditions, stable ECL intensity was obtained. This detector has been used in HPLC-ECL for the determination of multiple target fluoroquinolone residues in milk. There is no post column reagent addition, which would dilute the analytes, potentially leading to chromatographic band-broadening. The system is very simple with low dead volume, low baseline and background noise, together with high sensitivity and stability. The as-prepared ECL detector, when was used for the determination of ofloxacin, pefloxacin, enrofloxacin and difloxacin in milk, demonstrated adequate sensitivity to allow quantification of trace FQ levels in commercial milk samples. One or more of the target FQ analytes were present at levels above the LOD of the new ECL detector in each and every one of the 22 milk samples analysed.

## 1. Introduction

Quinolones are broad-spectrum synthetic antimicrobial agents used in the treatment of livestock and in aquaculture [1]. Their second generation drugs, fluoroquinolones (FQs), have been increasingly used in veterinary applications owing to enhanced antibacterial activities against gram-positive and gram-negative organisms [2]. Due to their possible abuse or misuse that may lead to public health problems, FQs were prohibited for use in food-producing animals in the USA [3], the European Commission in Council Regulation 2377/90/EC [4], the Japanese Positive List System [5] and Ministry of Agriculture of the People's Republic of China [6]. These organizations have all set maximum residue limits (MRLs) for FQs in milk and other food products in their respective territories. The MRL established for these FQs ranges is between 30 $\mu g\,kg^{-1}$ for (danofloxacin) and 100 $\mu g\,kg^{-1}$ for (enrofloxacin and ciprofloxacin) [7].

The most commonly employed methods to determine FQs are based on liquid chromatography – with different detection techniques; mainly UV [8], fluorescence [9,10], mass spectrometry (MS) [11–13] and chemiluminescence (CL). When UV and fluorescence detection was used, the sensitivity for FQs was limited. MS detection can offer high sensitivity for the determination of FQs, but it requires highly sophisticated apparatus and higher operating costs. Chemiluminescence (CL) detection has become an attractive detection method due to higher sensitivity, wide linear dynamic ranges and simpler instrumentation. CL methods coupled with HPLC and capillary electrophoresis (CE) for simultaneous analysis of FQs have been reported [14–16]. Compared with CL methods coupled with flow injection analysis, These methods have solved the separation problem, however, they are based on post-column addition of CL reagents, which has the shortcoming of analyte dilution effect and potentially resultant band broadening. There are also related cell dead volume and stability issues with the post-column addition approach to ECL detection. This is likely one of the main reasons that commercial chemiluminescence detectors are not yet commonplace in HPLC analysis.

Tris(2,2-bipyridyl)ruthenium(II) ($Ru(bpy)_3{}^{2+}$) ECL, based on electrogenerated $Ru(bpy)_3{}^{3+}$, has been used for HPLC detection in other studies. The electrogenerated $Ru(bpy)_3{}^{3+}$ further reacts with the analyte, producing light emission. However, its widespread applications are limited by the requirement to continuously deliver excess concentrations of $Ru(bpy)_3{}^{2+}$ into the reaction zone. Using this approach, $Ru(bpy)_3{}^{2+}$ is consumed, which implies high cost and

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pollution [17,18]. Since $Ru(bpy)_3{}^{2+}$ can be electrochemically recycled, this problem can be overcome by immobilizing $Ru(bpy)_3{}^{2+}$ on an electrode surface. Several different approaches have been used to immobilize $Ru(bpy)_3{}^{2+}$ or its derivatives on a variety of different electrode surfaces, such as, immobilization of $Ru(bpy)_3{}^{2+}$ in Nafion films [19] or by sol–gel techniques [20,21], direct attachment to an electrode by Langmuir–Blodgett techniques [22] or self-assembly [23]. Once $Ru(bpy)_3{}^{2+}$ is immobilized, several advantages are achieved, including saving the expensive reagent, enhancing the ECL signal and simplifying the experimental design, etc. [24].The above-mentioned designs were seldom used as a HPLC detector due to the poor stability of the output signal.

In this work, we designed a simple and stable HPLC detector for $Ru(bpy)_3{}^{2+}$ ECL. It was based on $Ru(bpy)_3{}^{2+}$ immobilized on a Pt wire surface with Nepem-105D, which is a perfluorinated ion exchange solution. Nepem-105D has branched chain perfluorinated vinyl ethers structure on a skeletal structure similar to that of PTFE. Since C–F bond length is short, the bond energy is high (486 kJ/mol) and electron-rich fluorine atoms are strongly electronegative, resulting in mutual repulsion between adjacent fluorine atoms and fluorine atoms along the zigzag polymer C–C chain spiral distribution. Also because the fluorine atomic volume is bigger than the hydrogen atom, it provides better coverage of the C–C chains, forming a low surface free energy fluoride protection layer. As Nepem-105D has good thermal stability, chemical stability and high exchange capacity, lots of $Ru(bpy)_3{}^{2+}$ can be exchanged through the ion-exchange process and electrostatic adsorption. Immobilized $Ru(bpy)_3{}^{2+}$could not migrate into the electro-inactive hydrophobic region with time, and it is also not destroyed upon exposure to mobile phases containing high organic content. This detector has been used in HPLC-ECL for determination of fluoroquinolone multiresidues in milk. There is no post-column reagent addition, so samples were not diluted, and band-broadening did not occur. The system is very simple with high sensitivity, good stability together with lower baseline and background noise levels than seen with comparative HPLC detector types.

## 2. Experimental

### 2.1. Chemical and reagents

Tris(2,2′-bipyridyl)dichlororuthenium(II) ($Ru(bpy)_3Cl_2{\cdot}6H_2O$), pure power of ofloxacin (OFL), enrofloxacin (ENR), pefloxacin (PEF), difloxacin (DIF), enoxacin (ENO), pipemidic (PIP), norfloxacin (NOR), ciprofloxacin (CIP), fleroxacin (FLE), gatifloxacin (GAT) and sarafloxacin (SAR) were purchased from Sigma (St. Louis, MO, USA). Nepem-105D perfluorinated ion exchange solution was purchased from BEST Industry & Trade Co., Ltd., Beijing, China. Acetonitrile, methanol and ethanol were of HPLC grade (Kermel Chemical Reagent Co., Ltd., Tianjin, China). Acetic acid (Yaohua Chemical Reagent Co., Ltd., Tianjin, China), sodium acetate (Xi'an Chemical Reagent Factory, Xi'an, China), and water for all solutions were purified using Akup-III-20 water purification system (Youpu Super Water Co., Ltd., Chengdu, China). All the other reagents were of analytical grade unless specified otherwise. The solutions used throughout the experiments were all passed through 0.22 μm filters before being injected into the HPLC system.

### 2.2. Stock solutions

Stock solutions of FQs were prepared by dissolving 5.0 mg OFL, PEF, CIP, PIP, SAR, and DIF in 10.0 ml methanol and dissolving 5.0 mg ENR, NOR, ENO, FLE, and GAT in 10.0 ml mixed solution of methanol and 10% acetic acid (9:1, v/v), respectively. The stock solutions were kept in the freezer at −4 °C. Working solutions were prepared daily by serial dilution of the stock solution with the mobile phase before use. Tris(2,2-bipyridine)ruthenium(II)chloride hexahydrate ($Ru(bpy)_3Cl_2{\cdot}6H_2O$) was prepared in our laboratory. A 10.0 mg $ml^{-1}$ $Ru(bpy)_3{}^{2+}$ solution was prepared by dissolving 50.0 mg of $Ru(bpy)_3Cl_2{\cdot}6H_2O$ in 5.0 ml water and was kept in a brown volumetric flask at −4 °C.

### 2.3. Apparatus

The HPLC-ECL is shown in Fig. 1A. The HPLC assembly consisted of a 1525 pump (Waters, USA) equipped with a syringe-loading sample injector valve (20 μl loop) and an Atlantis T3 column (I.D. 250 mm × 4.6 mm, HILIC Silica, 5 μm, 100 Å, Waters, USA). An electrochemical flow-cell (Fig. 1B) was used for the ECL experiments. The constant current was supplied by a YL4001A-105 invariable electrical current device (Yiliang Electric Instrument Plant, Beijing, China). The flow-cell consisted of a capillary tube and two Pt wires (Ø 0.4 mm), one as working electrode attached to the flow-cell inlet, the other was counter electrode attached to the flow-cell outlet. The flow-cell as placed close to the window of the photomultiplier tube. Data collection and processing were performed using an IFFL-D Flow Injection chemiluminescence Analyzer (Xi'an Remax Electronic Science-Tech Co. Ltd., Xi'an, China).

### 2.4. Preparation of the ECL detector

Pt wire was burned thoroughly and immersed into the mixed solution of $H_2SO_4$ and $H_2O_2$ (3:1, v/v) for 24 h and rinsed with deionized water before use. The preparation procedure of the ECL detector was as follows: Firstly, a Pt wire was immersed into 2% (v/v) ion-exchange solution of Nepem-105D in ethanol for 10 s, then it was dried at 80 °C, a thin film was formed on the surface of the Pt wire. Secondly, this Pt wire was put into a solution containing 10 mg $ml^{-1}$ $Ru(bpy)_3{}^{2+}$ for 2 min. $Ru(bpy)_3{}^{2+}$ was adsorbed into the film through an ion-exchange process and electrostatic adsorption, then it was dried at 80 °C again. Lastly, it was inserted into a capillary tube (I.D. 1.0 mm) as working electrode, then another Pt wire was inserted into this capillary tube as counter electrode, then the capillary tube was sealed by Teflon tape. The gap between two Pt wires was 1.0 mm. The length of capillary tube was 5.0 cm.

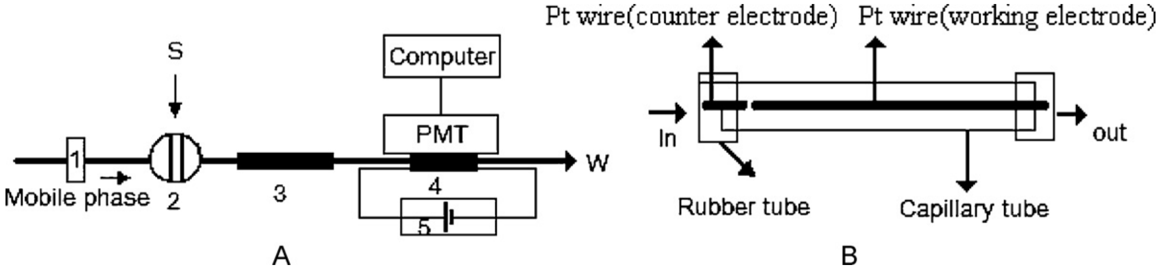

**Fig. 1.** Schematic diagram of HPLC-ECL detector of FQs (A). The structure of flow-cell (B). (1) Reciprocating pump; (2) injector; (3) HPLC column; (4) flow-cell; (5) invariable electrical current device; (S) sample solution; (W) waste; PMT: photomultiplier tube.

### 2.5. Preparation of milk samples

Milk samples were purchased from a local supermarket. Sample treatment was performed as follows.0.5 ml 10% $HClO_4$ was added to 0.5 ml aliquot of milk sample and vortex-mixed for about 60 s. After centrifugation at 15000 rpm for 3 min to remove precipitate, 20 μl aliquot of the supernatant was injected into the HPLC.

### 2.6. Analytical procedure

The mobile phase of 10 mM acetic acid–sodium acetate buffer (pH 5.4)/acetonitrile (75:25, v/v) was delivered at flow rate of 1.0 ml $min^{-1}$ to the HPLC column and the flow-cell successively. The invariable electrical current device (current: 30 μA) was switched on and the detector was equilibrated for 30 min. When a stable baseline was achieved, 20 μl of standard or sample solution was injected into the mobile phase. The quantitative determination was based on the ECL intensity *Is*, where *Is* is the ECL intensity (peak heights) in the presence of FQs.

## 3. Results and discussion

### 3.1. Optimization of ECL detector preparation conditions

An ECL detector was prepared according to the procedure described in Section 2.3 and characterized using OFL as a model analyte. The concentration of Nepem-105D ion-exchange solution, time of Pt immersed into Nepem-105D, the amount of immobilized $Ru(bpy)_3{}^{2+}$, the length and the diameter of capillary tube and the gap between the two Pt wires were optimized. Each parameter was considered to have an optimal value when a maximum in the signal intensity obtained from repeat injections of OFL standard solution was observed.

#### 3.1.1. Effect of the concentration of Nepem-105D ion exchange solution and $Ru(bpy)_3{}^{2+}$

In this work, Nepem-105D perfluorinated ion exchange solution was selected to immobilize $Ru(bpy)_3{}^{2+}$. After the Pt wire was immersed in the ion exchange solution and dried, a thin film formed on the surface of the Pt wire, when this Pt wire was immersed into a solution containing $Ru(bpy)_3{}^{2+}$, $Ru(bpy)_3{}^{2+}$ was adsorbed into the film through ion-exchange process and electrostatic adsorption. The film directly affected the immobilized $Ru(bpy)_3{}^{2+}$ and consequently affected the intensity of ECL output signals. The original concentration of ion-exchange solution was 5% (v/v), Pt wire was immersed into the original solution, the detector needs much more time to equilibrate. If the ion exchange solution was diluted with ethanol appropriately, the ECL intensity did not decrease (Fig. 2A).

According to the mechanism of $Ru(bpy)_3{}^{2+}$ ECL, $Ru(bpy)_3{}^{2+}$ is oxidized to form $Ru(bpy)_3{}^{3+}$, which further reacts with analyte, accompanied by light emission. Previous investigation of the ECL of $Ru(bpy)_3{}^{2+}$ immobilized in Nafion film has indicated that ECL could not be generated in any areas within the Nafion film where $Ru(bpy)_3{}^{2+}$ did not directly contacted the electrode[25]. So the amount of $Ru(bpy)_3{}^{2+}$ that directly contact the electrode affected the ECL intensity. As shown in Fig. 2B, when the Pt wire coated with surface ion exchange film was immersed into 10 mg $ml^{-1}$ $Ru(bpy)_3{}^{2+}$ solution for 2 min, the highest ECL intensity was obtained.

#### 3.1.2. Effect of the length and diameter of capillary tube

In this study, glass tubes with different lengths and diameters were used. The length and diameter of tube did not affect peaks shape, however, they all affected ECL intensity. Glass tube with small diameter and long length was optimal. ECL intensity decreased with the diameter increasing and increased with length increasing. capillary tube with 1.0 mm inside diameter and 5 cm in length was chosen for the quantitative assay experiments described.

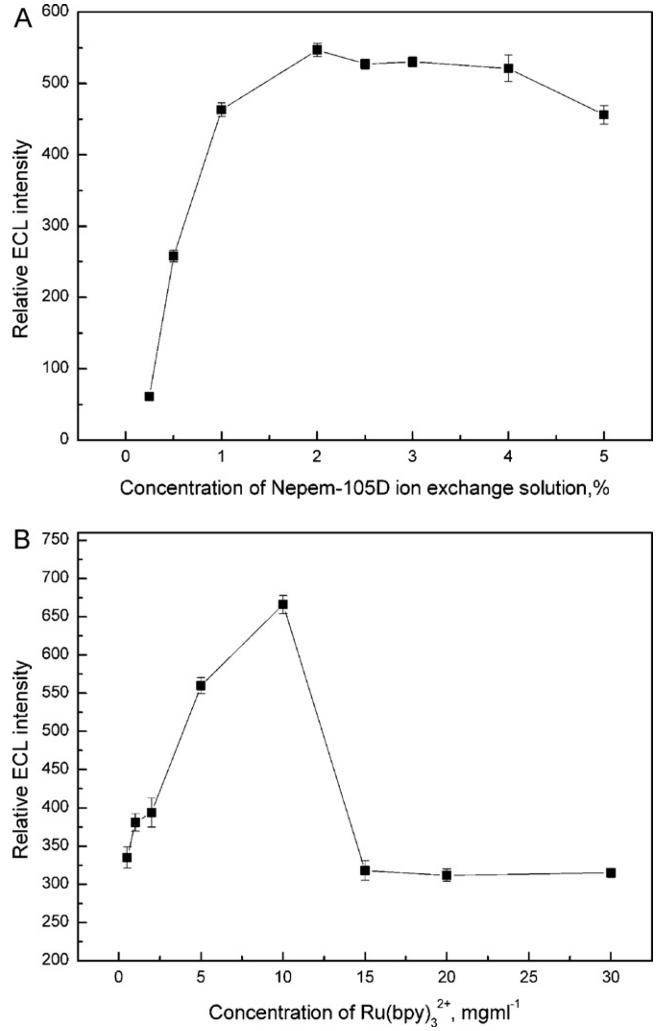

**Fig. 2.** Effect of the concentration of Nepem-105D ion exchange solution (A) and $Ru(bpy)_3{}^{2+}$ (B) on ECL intensity of OFL (6.0 μg $ml^{-1}$).

#### 3.1.3. Effect of the gap between electrodes

When the gap between the two electrodes was not too large, from 0.5 mm to 3.0 mm, the ECL response to OFL was quite constant, but when it exceeded 3.0 mm, the ECL response diminished markedly with increasing gap width. In this assay, a 1.0 mm gap was selected.

#### 3.1.4. Stability of the ECL detector

Under optimal conditions, stable ECL intensity was obtained (Fig. 3). The intra-day precision of six repeat injections of 0.3 μg $ml^{-1}$ OFL solution was 3.6% and the inter-day precision of the method was 6.8% from the analysis of the same sample on three consecutive days. After the ECL detector was kept at room temperature for about 3 months, the potential was essentially unchanged and ECL intensity decreased by only 9.7% compared with the initial steady state value, the result suggested that the new detector described here had good stability.

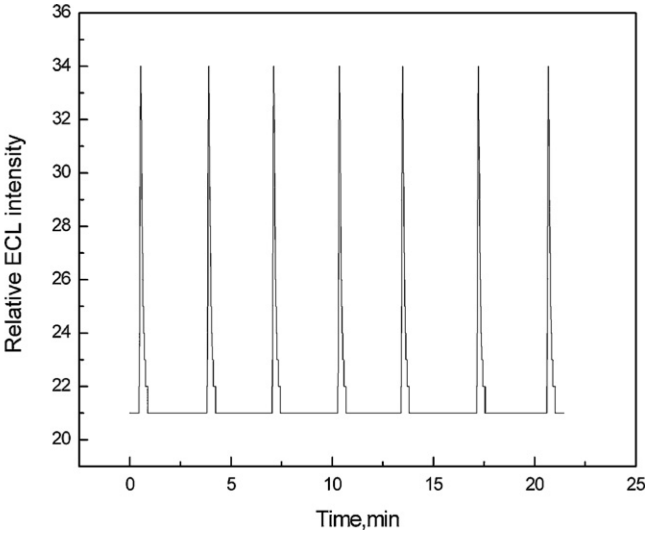

**Fig. 3.** The stability of OFL (0.3 μg ml$^{-1}$) ECL intensity. Detector preparation conditions are described in Section 2.3. The mobile phase was a 10 mM acetic acid–sodium acetate buffer (pH 5.4)–acetonitrile (75:25, v/v), flow rate: 1.0 ml min$^{-1}$, current intensity: 30 μA. injection volume: 20 μl.

### 3.2. Detection of FQs

As we know, the detection of fluoroquinolone residues in animal food is very important. In order to characterize the proposed detector, coupled with HPLC separation, the detector was trialled for the determination of OFL, ENR, PEF, and DIF residues in milk.

In the HPLC-ECL experiment, parameters that affected the determination were optimized. As shown in Fig. 4A, the ECL of Ru(bpy)$_3$$^{2+}$/FQs was very sensitive to the solution pH. The effect of solution pH was examined over the range of 4.0–5.8. From 4.0 to 5.8, separation of four FQs became better, but the solution pH had a varying effect on each FQ. Maximum ECL intensity was observed at pH 4.2 for OFL and PEF, but that pH 5.4 for ENR and DIF. Considering the separation and the pH tolerance of the HPLC column, pH 5.4 was selected the optimal pH for this assay.

The isocratic mobile phase was 10 mM acetic acid–sodium acetate buffer (pH 5.4)/acetonitrile (75:25, v/v), and the concentrations of OFL, PEF, ENR and DIF standards were 6.0 μg ml$^{-1}$, 10.0 μg ml$^{-1}$, 6.0 μg ml$^{-1}$ and 20.0 μg ml$^{-1}$, respectively.

The concentration of electrolyte also influenced the ECL intensity of Ru(bpy)$_3$$^{2+}$/FQs, but its effect was less than that of the pH. The effect was investigated from 5 to 40 mM, the ECL intensity increased with increasing electrolyte concentration. As higher concentrations of electrolyte are harm for the HPLC column, 10 mM was selected as a compromise. The results are shown in Fig. 4B.

The ECL intensity and HPLC separation of the target FQs were highly dependent on the choice of solvent used as the organic component of the mobile phase. Methanol and acetonitrile are commonly used in mobile phases, so acetonitrile and methanol have been evaluated as the organic part of the mobile phase in our experiment. Acetonitrile and methanol can be used in this study, however, compared with methanol, higher ECL intensity was obtained when acetonitrile was used, acetonitrile was therefore chosen for further study. The concentration of acetonitrile in the mobile phase was optimized by varying the acetonitrile–10 mM acetic acid–sodium acetate buffer (pH 5.4) composition in the range of 15–45%. As shown in Fig. 4C, the separation of the FQs became poor with more acetonitrile, but the ECL intensity became higher for ENR and DIF. Maximum ECL intensity for OFL and PEF was observed at 40%. When the mobile phase contained 30% acetonitrile, the target FQs were not well separated. When the mobile phase contained 25% acetonitrile, the FQs were well-separated with good sensitivity, even though the ECL intensities were slightly lower than maximum values. To maintain good chromatographic separation, 25% acetonitrile was used for the following experiments. Standard analyte concentrations were the same as those used to determine optimum pH.

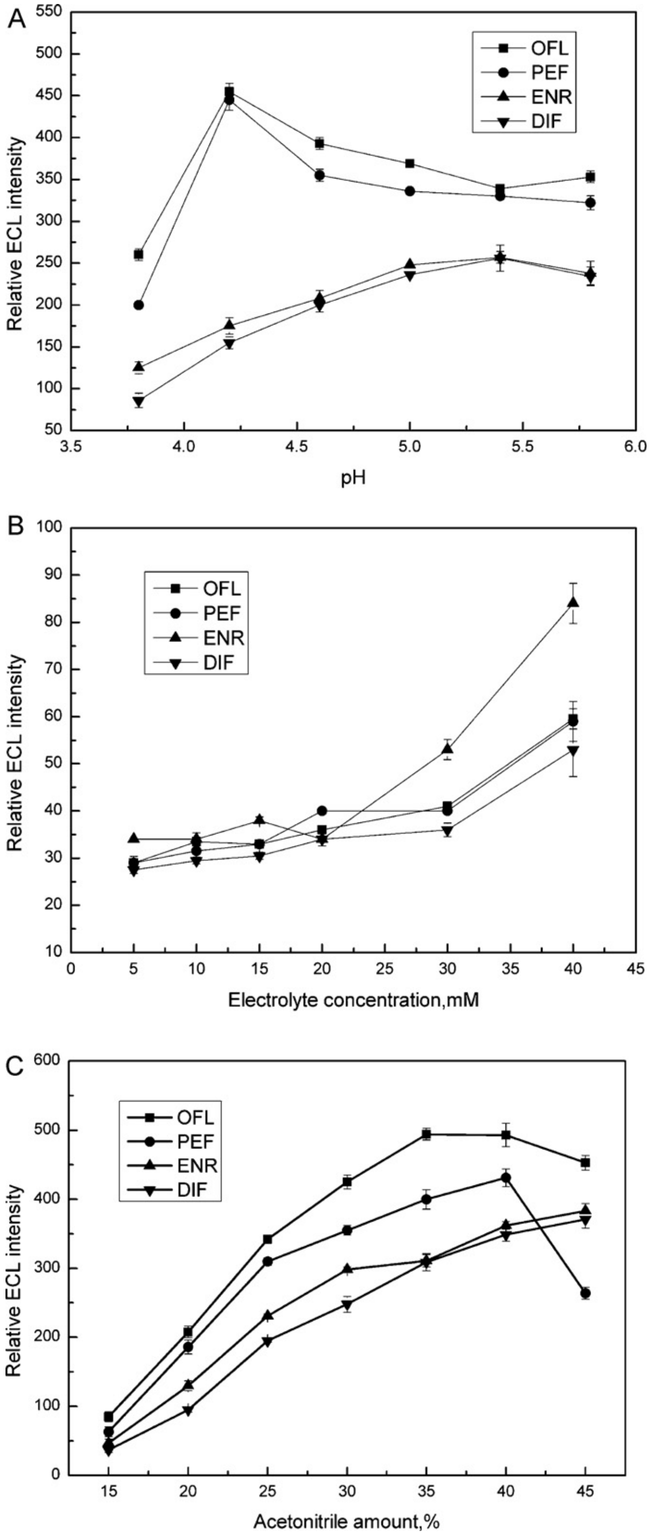

**Fig. 4.** Effect of mobile phase pH (A), the electrolyte concentration (B) and the amount of acetonitrile (C) on ECL intensity. Flow rate: 1.0 ml min$^{-1}$, current intensity: 30 μA. injection volume:20 μl. In this experiment, the amount of acetonitrile was 25%, the concentrations of OFL, PEF, ENR and DIF were 0.6 μg ml$^{-1}$, 1.0 μg ml$^{-1}$, 0.6 μg ml$^{-1}$ and 2.0 μg ml$^{-1}$, respectively.

**Table 1**
The regression equation, correlation coefficient and the detection limits.

| | The regression equation | Correlation coefficients ($R^2$) | Liner range ($\mu$g ml$^{-1}$) | The detection limits. ($\mu$g ml$^{-1}$)[a] |
|---|---|---|---|---|
| OFL | $y = 103.2x + 20.99$ | 0.9984 | 0.02–4.0 | 0.006 |
| PEF | $y = 34.07x + 19.81$ | 0.9994 | 0.05–10.0 | 0.02 |
| ENR | $y = 33.54x + 21.74$ | 0.9998 | 0.06–12.0 | 0.02 |
| DIF | $y = 16.28x + 17.00$ | 0.9986 | 0.20–20.0 | 0.06 |

[a] The detection limit was calculated at a signal-to-noise ratio of 3.

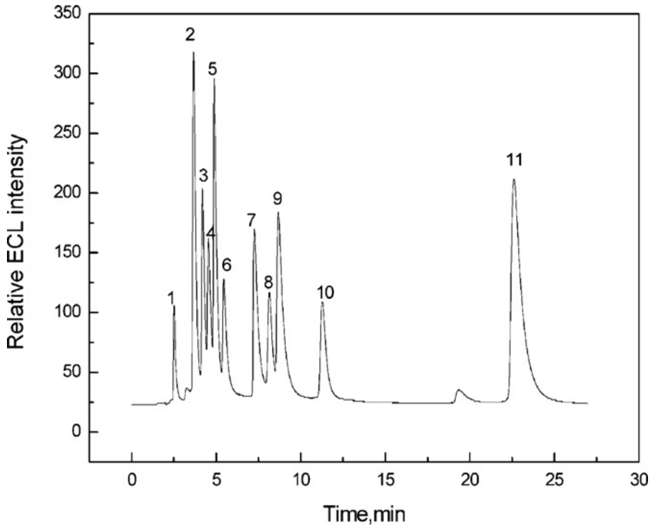

**Fig. 5.** Typical chromatograms of 11 kinds of FQs: (1) ENO ($3.2 \times 10^{-5}$ g ml$^{-1}$); (2) PIP ($3.6 \times 10^{-5}$ g ml$^{-1}$); (3) NOR ($1.0 \times 10^{-5}$ g ml$^{-1}$); (4) CIP ($2.4 \times 10^{-5}$ g ml$^{-1}$); (5) OFL ($3.0 \times 10^{-6}$ g ml$^{-1}$); (6) FLE ($1.0 \times 10^{-5}$ g ml$^{-1}$); (7) GAT ($2.0 \times 10^{-5}$ g ml$^{-1}$); (8) PEF ($6.0 \times 10^{-6}$ g ml$^{-1}$); (9) SAR ($7.0 \times 10^{-5}$ g ml$^{-1}$); (10) ENR ($7.0 \times 10^{-6}$ g ml$^{-1}$); and (11) DIF ($2.0 \times 10^{-5}$ g ml$^{-1}$). In this experiment, the mobile phase was a 10 mM acetic acid–sodium acetate buffer (pH 5.4)–acetonitrile (80:20, v/v), flow rate: 1.0 ml min$^{-1}$, current intensity: 30 $\mu$A. injection volume: 20 $\mu$l.

### *3.3. Linearity and detection limit of FQs*

Under the selected conditions, the calibration graph of ECL intensity versus FQs concentration was linear in the range of 0.02–4.0 $\mu$g ml$^{-1}$, 0.05–10.0 $\mu$g ml$^{-1}$, 0.06–12.0 $\mu$g ml$^{-1}$ and 0.20–20.0 $\mu$g ml$^{-1}$ for OFL, PEF, ENR and DIF, respectively. The detection limit (LOD) was calculated at a signal-to-noise ratio of 3, while limit of quantification (LOQ) value was calculated by using a signal-to-noise ratio of 10. The regression equation, correlation coefficient and the detection limits are listed in Table 1.

### *3.4. Application to milk sample*

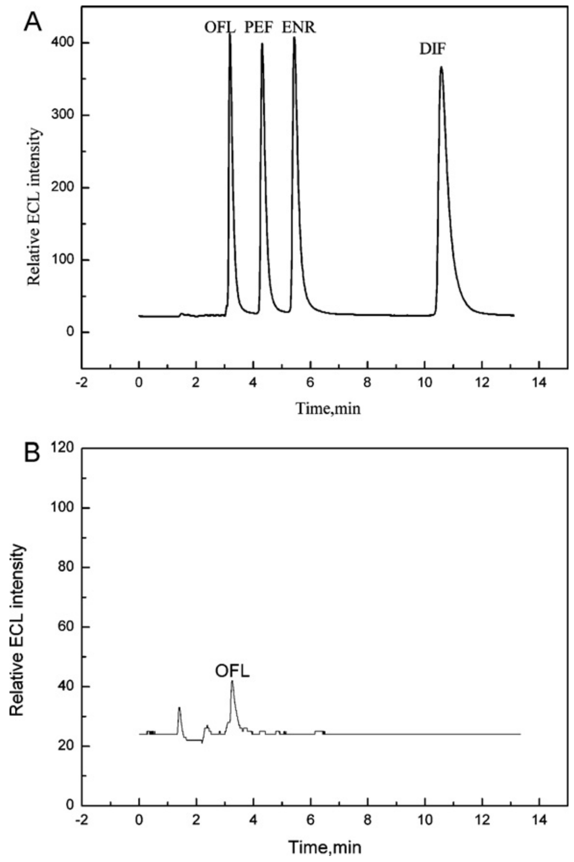

**Fig. 6.** Chromatogram of spiked sample (A) and a milk sample (B). Peaks: OFL (3.19 min); PEF (4.60 min); ENR (5.87 min); DIF (11.47 min). HPLC conditions are described in Section 2.6.

Fig. 5 shows the chromatograms of 11 FQs using contain 20% acetonitrile as a mobile phase. The HPLC separation of OFL, PEF, ENR and DIF residues in milk were performed isocratically at a flow-rate of 1.0 ml min$^{-1}$ and the eluate from the column was continuously monitored by ECL. Representative chromatograms of a spiked milk sample (Fig. 6A) and a milk sample found to contain OFL (0.17 $\mu$g ml$^{-1}$) (Fig. 6B) are shown. The recovery tests were carried out on spiked samples, and the results are shown in Table 2. In

**Table 2**
Recovery for FQs at different spiked levels in milk samples.

| | Sample ($\mu$g ml$^{-1}$) | Spiked ($\mu$g ml$^{-1}$) | Found ($\mu$g ml$^{-1}$)[b] | Recovery (%)[c] | RSD (%) |
|---|---|---|---|---|---|
| OFL | 0.04 | 0.20 | 0.24 ± 0.003 | 97.9 | 1.7 |
| | | 0.80 | 0.80 ± 0.034 | 94.6 | 4.5 |
| PEF | ND[a] | 0.50 | 0.50 ± 0.017 | 100.8 | 3.2 |
| | | 2.00 | 2.00 ± 0.099 | 99.9 | 5.0 |
| ENR | ND | 0.60 | 0.60 ± 0.013 | 97.9 | 1.7 |
| | | 2.40 | 2.31 ± 0.105 | 96.4 | 4.6 |
| DIF | ND | 1.00 | 0.95 ± 0.022 | 95.3 | 2.3 |
| | | 4.00 | 3.78 ± 0.099 | 94.5 | 2.6 |

[a] Not detected.
[b] Results expressed as the average value of six determinations ± standard deviation.
[c] Results were the average value of six determinations.

**Table 3**
Results of determination of FQs in milk.

| Sample no. | Detected concentration ($\mu g\,ml^{-1}$) | | | |
|---|---|---|---|---|
| | OFL | PEF | ENR | DIF |
| 1 | 0.04 | ND[a] | ND | ND |
| 2 | 0.04 | ND | ND | ND |
| 3 | 0.05 | ND | ND | ND |
| 4 | 0.02 | ND | ND | ND |
| 5 | 0.37 | 1.12 | 1.20 | 1.38 |
| 6 | 0.46 | 1.42 | 1.50 | 1.84 |
| 7 | 0.17 | ND | ND | 2.40 |
| 8 | 0.03 | 0.59 | 0.90 | ND |
| 9 | ND | 3.20 | 0.10 | ND |
| 10 | ND | 0.060 | ND | ND |
| 11 | ND | 2.32 | ND | ND |
| 12 | 0.67 | 1.74 | 1.87 | 3.84 |
| 13 | 0.99 | 2.49 | 2.89 | 5.28 |
| 14 | 1.59 | 3.32 | 4.06 | 6.97 |
| 15 | 1.88 | 4.64 | 5.18 | 9.03 |
| 16 | 0.10 | 0.39 | 0.28 | 0.74 |
| 17 | 0.04 | ND | ND | ND |
| 18 | 0.03 | 0.06 | ND | ND |
| 19 | 0.11 | 0.32 | 0.21 | 0.79 |
| 20 | 0.21 | 0.56 | 0.52 | 1.27 |
| 21 | 0.30 | 0.80 | 0.79 | 1.77 |
| 22 | 0.39 | 0.99 | 1.09 | 2.20 |

[a] Not detected.

addition, 22 milk samples were analysed using the detector. Results are shown in Table 3.

## 4. Conclusion

In this work, a simple, stable and sensitive ECL detector was developed. The proposed detector was based on $Ru(bpy)_3^{2+}$ immobilized on a Pt wire surface with Nepem-105D ion exchange solution. The flow-cell consisted of only two Pt wires and a capillary tube, which made this detector very simple. Coupled with HPLC separation, the detector has been used for the determination of ofloxacin, pefloxacin, enrofloxacin and difloxacin residues in milk. The system has a small dead volume, and much lower baseline and background noise than comparative HPLC detectors used for monitoring FQs. The results show that application of the detector to analyse fluoroquinolone residues in milk is feasible.

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