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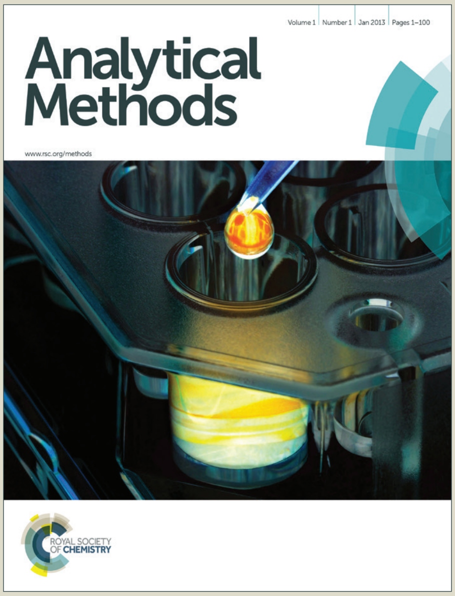

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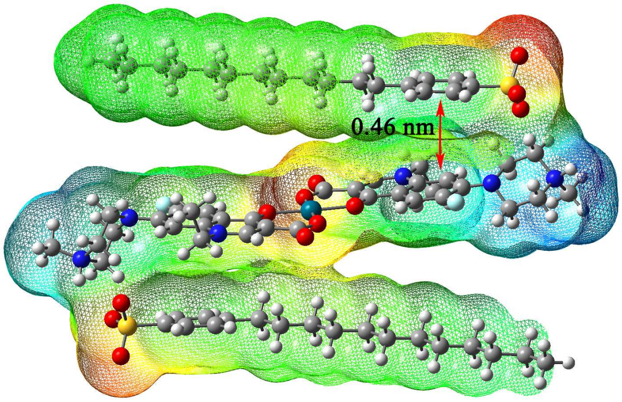
Graphical Abstract



The structure of ternary complexes, Jian Wang, Ling Kong, Wei Shen, Xiaoli Hu, Yizhong Shen, Shaopu Liu

Synergistic fluorescence quenching of quinolone antibiotics by palladium(II) and sodium dodecyl benzene sulfonate and the analytical application

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Abstract

In weakly acidic and neutral media, palladium(II) or sodium dodecyl benzene sulfonate (SDBS) can separately quench the fluorescence of quinolone antibiotics (FLQs) in varying degrees. When Pd(II) react with SDBS and FLQs to form ternary complexes, a enhanced fluorescence quenching of FLQs could be observed. This synergistic fluorescence quenching effect has high sensitivity for Pd(II) detection and the detection limit could reach to 0.13 ng.mL^{-1}. Based on this, a rapid, simple and reliable method for the determination of Pd(II) in aqueous samples was established. The optimum reaction conditions of the method were tested. The rection information of FLQs-SDBS-Pd(II) system was investigated by absorption spectra, fluorescence spectra, and was calculated by quantum chemical using density function theory B3LYP under polarizable continuum model (PCM). Results showed that pefloxacin (PEF) molecule exists as zwitter-ion of HL$^{\pm}$ reacting with Pd(II) to form plane

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bis(PEF)Pd chelates, which further binds two SDBS molecules to form ternary complexes, the composition of Pd(II)-PEF-SDBS complex was found to be 1:2:2. The ternary complexes resulted in higher fluorescence quenching efficiency and enhanced the sensitivity for the determination of Pd(II).

Keywords: fluorescence quenching, quinolone antibiotics, Pd(II), sodium dodecyl benzene sulfonate

1. Introduction

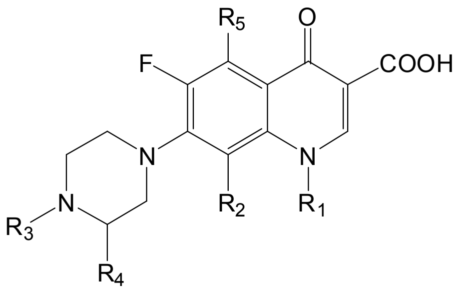
Quinolone antibiotics, a class of inexpensive antibiotics, are widely used in clinical treatment. They can affect or improve their biological and antimicrobial acitivity by binding some metal ions to form complexes. Therefore, the interaction between quinolone antibiotics and metal ions has aroused extensive concern. Their interactions with Cu(II) [1,2], Zn(II) [3,4], Co(II) [5], Cd(II) [6,7], Al(III) [8] Fe(III) [9-10], Ni(II) [11], Mg(II) [2], W(VI) [12], V(V) [12] and some rare earth ions [13-15] , have been researched. But their analytical application potential has less been focused.

Presently, palladium catalysts are widely used to reagents in organic synthesis, hazardous palladium is often found in reaction products even after purifications. Thus, the detection of palladium is an important process in the production of fine chemicals and active pharmaceutical ingredients. The detection methods of palladium include spectrophotometry [16-18], flame atomic absorption spectroscopy method (FASS) [19-26] and ICP-atomic emission spectroscopy method (ICP-AES) [27-28]. The fluorescence detection method [29-30] is also one of important method.

We found that, in weakly acidic and neutral media, FLQs showed stronger fluorescence, which could decrease in some different extents while there was the presence of Pd(II) or SDBS. When both Pd(II) and SDBS exist，synergistic effect of fluorescence quenching can be observed, causing greater decrease of fluorescence intensity of FLQs. This resulted from the formation of Pd(II)-FLQs-SDBS ternary

complexes. Therefore, the ternary complexes have better analytical application potential.

In this work, the fluorescence spectral characteristics of four kinds of quinolone antibiotics including pefloxacin (PEF), levofloxacin (LEV), lomefloxacin (LOM) and fleroxacin (FLE) were described. Their structures are shown in Fig.1. The formation of the Pd(II)-FLQs and SDBS-FLQs binary complexes as well as Pd(II) -FLQs-SDBS ternary complexes could quench the fluorescence of FLQs. The quenching efficiency of the ternary complexes was higher than the sum of those of two binary complexes. The Pd(II)-PEF-SDBS system had the highest sensitivity for Pd(II) and the limit of detection could reach to 0.13 ng.mL^{-1}. The spectral characteristics of the fluorescence spectra were investigated. Taking it as an example, the optimum reaction conditions and selectivity of the method were tested. The results showed the method had good selectivity. A rapid, simple and reliable method for determination of Pd(II) in environmental aqueous samples based on the formation of Pd(II)-PEF-SDBS ternary complexes, was established.



	R_1	R_2	R_3	R_4	R_5
PEF	C_2H_5	H	CH_3	H	H
FLE	$-CH_2CH_2F$	F	CH_3	H	H
LEV	$-OCH_2CH(CH_3)-$		CH_3	CH_3	H
LOM	C_2H_5	F	H	CH_3	H

Fig. 1 Structures of PEF, FLE, LEV and LOM

The type of fluorescence quenching was discussed via the absorption and fluorescence spectroscopies. Based on the changed absorption spectrum, the decreased k_{SV} values for increased temperatures, the synergistic quenching effect of Pd(II) and SDBS on FLQs fluorescence was a single static quenching event. Based on the results, the quantum chemical calculation was considered. All of the compounds were fully optimized by density function theory B3LYP [31] under polarizable continuum model (PCM). In processing, the pseudopotential basis set LANL2DZ was used for Pd and 6-31G* for other atom. The results showed that, PEF molecules existed as zwitter-ion $HL^{\pm}$ reacting with Pd(II) to form plane bis(PEF)Pd chelates, which further bound two SDBS molecules to form ternary ion associates via electrostatic attraction, hydrophobic force and aromatic ring stacking effect. The interaction contained both chelation and ion-association effects, making the ternary complexes more stable and increasing the fluorescence quenching efficiency. This created greater condition establishing a more sensitive fluorescence quenching method for the determination of Pd(II).

2. Experimental

2.1 Apparatus and reagents

A Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) was used to acquire the fluorescence spectra. Slit (EX/EM): 5.0 nm /5.0 nm, PMT voltage: 400 V. A pHS-3C meter (Shanghai precision & scientific instrument Co., Ltd) was used to adjust the pH values.

The concentration of stock solution of fluoroquinolone antibiotics (Huamei biology technology Co. Ltd.), including pefloxacin (PEF), levofloxacin (LEV), lomefloxacin (LOM) and fleroxacin (FLE) was 100.0 $\mu g.mL^{-1}$. Their working solution concentrations were 10.0 $\mu g.mL^{-1}$.

Surfactant including Sodium dodecyl benzene sulfonate (SDBS), sodium dodecyl sulfonate (SLS) and sodium dodecyl sulfate (SDS) were obtained from Shanghai Reagent Factory, China. Their working solution concentrations were 5.0×10^{-3} $mol.L^{-1}$. A stock solution of Pd(II) (4.0×10^{-3} $mol.L^{-1}$) was prepared by weighing 0.1774 g of $PdCl_2$ (Shanghai Reagent Factory, China) into a 50 mL beaker and by adding 1.0 mL of concentrated hydrochloric acid. It was heated by water bath until all is dissolved, and then it was transferred into a 250.0 mL of flask, diluted to the mark and mixed. The working solution was further diluted with water to 4.0×10^{-6} $mol.L^{-1}$.

Britton-Robinson (BR) buffer solutions were used to control the pH of the interaction system, which were adjusted to 4.6-6.2 with the mixed acid (composed of 0.04 $mol.L^{-1}$ H_3PO_4, HAc and H_3BO_3) and 0.2 $mol.L^{-1}$ NaOH. The pH values were monitored with a pH meter. All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments.

2.2 General procedure

Into a 10.0 mL calibrated flask were added 1.0 mL of BR buffer solution, 2.0 mL of 10 μg.mL^{-1} fluoroquinolone antibiotics solution, 1.0 mL of 5.0×10^{-3} mol·L^{-1} SDBS and suitable amounts of Pd(II) solution. The mixture was then diluted with water to 10.0 mL and mixed thoroughly. After incubation for 5 min at room temperature, the fluorescence spectra of the solution were acquired. The decreased fluorescence intensity (ΔF) of the system was represented by $\Delta F = F_0 - F$, where F and F_0 were the fluorescence intensities of the complex and the reagent blank, respectively.

All of the compounds were fully optimized by density function theory B3LYP under polarizable continuum model (PCM) and considering the effects of solvent. In processing, the pseudopotential basis set LANL2DZ was used for Pd and 6-31G* for other atom. The reaction energy and structure of Pd(II)-PEF-SDBS complex were calculated by quantum chemical calculations by way of frozen bond length.

3 Results and discussion

3.1 Fluorescence spectra

The fluorescence spectra of four kinds of FLQs including PEF, FLE, LOM and LEV, and the Pd(II)-FLQs bidentate complex as well as the Pd(II)-FLQs-SDBS ternary complex, are shown in Fig.2. Their spectral characteristics are shown in Table 1. It can be seen that：(1) the four kinds of FLQs have similar spectral characteristics because they all have a large conjugated system, preferably planarity and rigidity, and have similar parent structures. But their maximum excitation (λ_{ex}) and emission wavelengths (λ_{em}) and fluorescence intensities have some difference owing to different groups in parent structure (see Fig. 1). Their maximum excitation wavelength and maximum emission wavelength were in the range of 276 nm ~ 293 nm and 442 nm ~ 487 nm, respectively. Compared to those of the three others, the LEV have different spectral characteristics, and its λ_{ex} and λ_{em} have red shifts 12 nm ~17 nm and 42 nm ~ 45 nm, respectively, because it has a branch chain of fluoroethane. In addition, their fluorescence intensity is different, which were in the order of PEF>FLE>LOM >LEV. (2) High concentration of SDBS can cause the decrease of fluorescence quenching of FLQs (the concentration of SDBS is 83 times than those of FLQs in Fig.2 and Table 1). Lower concentration of SDBS hardly quenches the fluorescence of FLQs. So this reaction has no actual analysis value. (3) The Pd(II) can cause obvious decrease of FLQs fluorescence. The reaction has higher sensitivity. The Pd(II) has the best quenching efficiency on PEF, followed by FLE, LOM and LEV in turn. (4) when FLQs react with Pd(II) and SDBS to form ternary complexes, the FLQs fluorescence are much more quenched. Its fluorescence quenching intensity (ΔF_3) is higher than the sum of those of Pd(II)-FLQs and SDBS-FLQs complexes (ΔF_1+ΔF_2). This indicated that the ternary complexes could produce a synergistic quenching effect，which could greatly decrease the detection limit for determination of Pd(II). Taking the greatest sensitive Pd(II)-PEF-SDBS system as an example, the optimum conditions, influencing factors, the analytical properties and reaction mechanism, have been discussed.

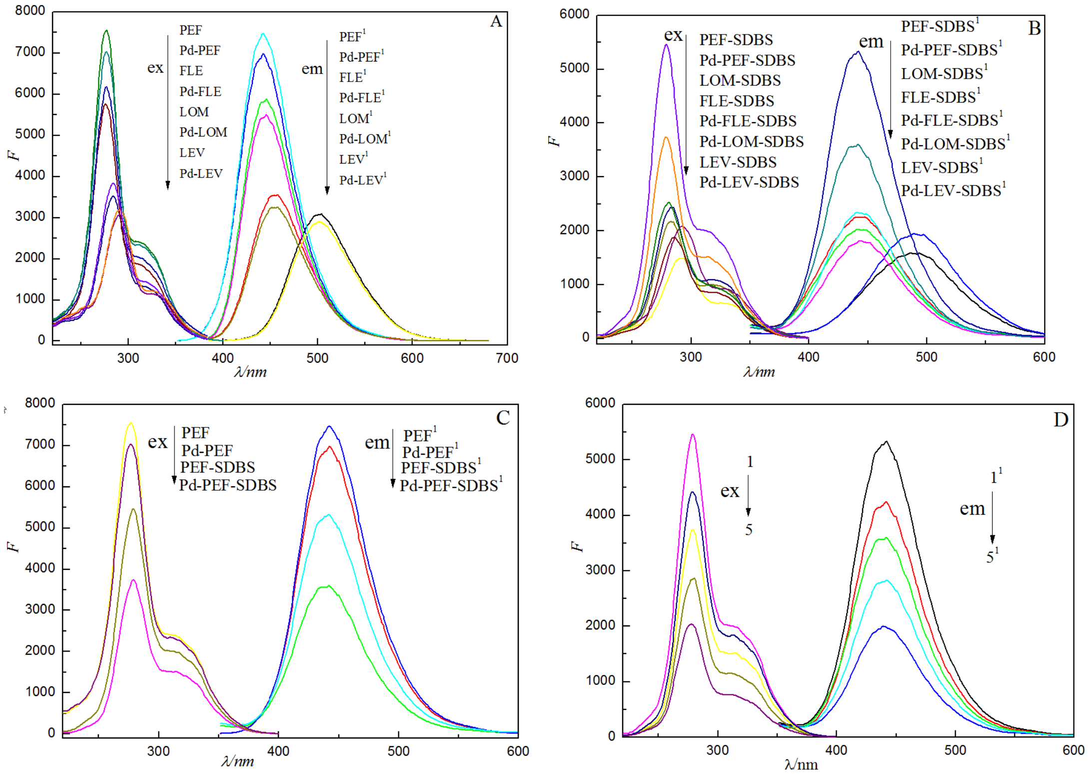


Fig. 2. Fluorescence spectra. A: Pd(II)-FLQs system, B: Pd(II)-FLQs-SDBS systems, C and D: Pd(II)-PEF-SDBS system, pH=5.6, concentration of FLQs is 2.0 $\mu g\cdot mL^{-1}$, concentration of SDBS is 5.0×10^{-4} $mol\cdot L^{-1}$, concentrations of Pd(II) in Fig.1 A, B, C are 4.0×10^{-7} $mol\cdot L^{-1}$, and those in Fig.1D from curve 1 to 5 are 0, 2.0×10^{-7}, 4.0×10^{-7}, 6.0×10^{-7} and 8.0×10^{-7} $mol\cdot L^{-1}$.

Table 1 Fluorescence spectral characteristics of Pd(II)-FLQs-SDBS systems

System	λex/λem (nm)	FLQs F_0	Pd(II)-FLQs F_1	ΔF_1 $(F_0\text{-}F_1)$	FLQs-SDBS F_2	ΔF_2 $(F_0\text{-}F_2)$	Pd(II)-FLQs-SDBS F_3	ΔF_3 $(F_0\text{-}F_3)$	$\Delta F_1+\Delta F_2$
LEV	293/487	2990	2906	84	1947	1043	1401	1579	1127
LOM	281/445	3598	3494	104	2339	1259	1814	1784	1363
FLE	276/443	6203	6063	140	2206	3997	1830	4373	4137
PEF	276/442	7486	7296	190	5329	2157	3599	3887	2347

3.2 Optimum experimental conditions

3.2.1 Effect of pH

Fig. 3 shows the effect of pH on the quenched fluorescence intensity (Δ*F*) of Pd(II)-FLQs-SDBS systems in the pH ranges of 3.0-7.0. The optimum pH ranges for all four systems, as expected, are about the same and range from 4.6~6.2. In this interval, ΔF values reach the maximum and kept consistent. When the pH is beyond this range, ΔF values were lower. Hence, pH 5.6 (1.0 mL) was chosen as the working pH.

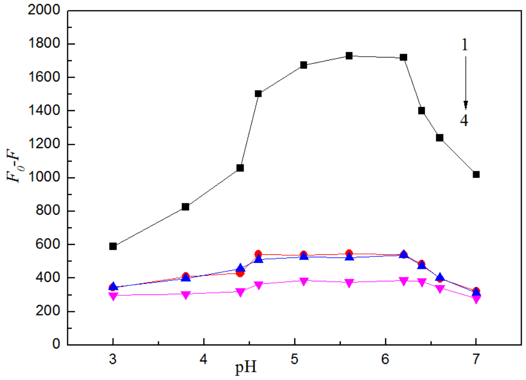


Fig. 3. pH on fluorescence of Pd(II)-FLQs-SDBS system. PEF concentration is 2.0 $\mu g \cdot mL^{-1}$, SBDS concentrations are 5.0×10^{-4} $mol \cdot L^{-1}$, Pd(II) concentration is 4.0×10^{-7} $mol \cdot L^{-1}$, from curve 1 to 4 are PEF, LEV, LOM and FLE systems, and their measurements are at themselves maximum excitation (*λex*) and emission wavelengths (*λem*), respectively.

3.2.2 Effect of FLQs concentration

Experimental results showed that when the concentration of FLQs was 2.0 $\mu g.mL^{-1}$. For lower than 2.0 $\mu g.mL^{-1}$, the ternary complex reaction was incomplete; for greater than 2.0 $\mu g.mL^{-1}$, F_0 values increased. In this experiment, the concentration FLQs was 2.0 $\mu g.mL^{-1}$.

3.2.3 Effect of anionic surfactant concentration

Effects of anionic surfactants, such as SDS, SDBS and SLS, on fluorescence of FLQs in anionic surfactants-FLQs binary complexes and Pd(II)-anionic surfactant-FLQs binary complexes were examined. The results showed that, SDS and SLS had no influence on fluorescence of FLQs in both binary and ternary complexes. Only SDBS could cause the decrease of the FLQs fluorescence, and result in obvious “synergistic quenching effect”. So, only SDBS was used in ternary complex reaction.

Taking Pd(II)-PEF-SDBS system as an example, the effect of SDBS concentration on fluorescence was investigated in the range of 0~5.0×10^{-3} mol.L^{-1}, and the results were shown in Fig.4. The quenched fluorescence intensity reached the maximum at the concentration range of 4.0~5.0×10^{-4} mol.L^{-1}, on the contrary they decreased with increasing the concentration of SDBS further. This indicates that: (1) lower concentration of SDBS causes incomplete formation of ternary complexes. When its concentration is in the range of 4.0~5.0×10^{-4} mol.L^{-1}, the ternary complexes form completely, resulting in the maximum decrease of fluorescence. (2) when the concentration of SDBS is lower than 5.0×10^{-4} mol.L^{-1}, which is far lower than its critical micelle concentration of 8.3×10^{-3} mol.L^{-1} [32], the SDBS is mainly in the form of one charged anionic monomer ($C_{12}H_{25}SO_3^-$). Therefore, it is still in monomer form in Pd(II)-PEF-SDBS ternary complexes. (3) higher concentration of SDBS will cause the formation of dimer, trimer, multimer or micelles. This will hinder the formation of ternary complexes, even cause the dissociation of ternary complexes to become binary complexes. Therefore this is not conducive to synergistic fluorescence quenching.

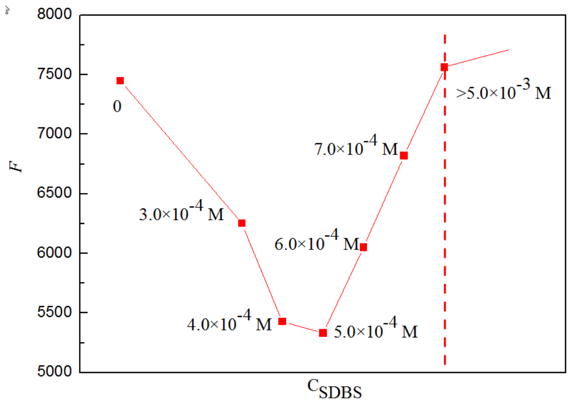


Fig. 4 Effect of SDBS concentration on fluorescence of Pd(II)-PEF-SDBS system. PEF concentration is 2.0 μg·mL^{-1}, SBDS concentrations are between 0 and 5.0×10^{-3} mol·L^{-1}, Pd(II) concentration is 4.0×10^{-7} mol·L^{-1}, pH5.6.

3.2.4 Reaction speed and the stability

At room temperature, the four kinds of ternary complexes reaction completed in 5 minutes and the quenched fluorescence intensity (Δ*F*) could remain constant for 12 hours.

3.3 Standard curve and the limits of detection

Under optimum conditions, Δ*F* values of ternary complexes and binary complexes (see Fig. 5.), were plotted versus the concentration of Pd(II). All the parameters of the standard curves and the limits of detection were listed in Table 2. The results show that the limits of detection for Pd(II) are in the range of 1.74~3.42 ng.mL^{-1} in binary complex systems. The Pd(II)-PEF system has the highest sensitivity and the limit of detection for Pd(II) is 1.74 ng.mL^{-1}. For ternary complexes systems, owing to the synergistic fluorescence quenching effect, have higher sensitivity. Their limits of detection for Pd(II) are between 0.13~0.67 ng.mL^{-1}, and that of the Pd(II)-PEF-SDBS system（0.13 ng.mL^{-1}）is 14 times higher that the corresponding binary system. And this method generally does not require pre-enrichment processing, so the method is simpler and faster than spectrophotometry [18-20], flame atomic absorption spectroscopy method [21-28] and ICP-atomic emission spectroscopy method (ICP-AES) [29-30].

Table 2 Related parameters of the calibration graphs and the detection limits

System	Mesurement wavelength $\lambda_{ex}/\lambda_{em}$(nm)	Linear regression equation (μg·mL^{-1})	Correlation coefficient(r)	Linear range /ng·mL^{-1}	Detection limits 3σ/ng·mL^{-1}
Pd(II)-LEV	293/487	ΔF=10.2+1.74×10^{3}c	0.9996	9.00~1060	2.65
Pd(II)-LOM	281/445	ΔF=52.3+1.24×10^{3}c	0.9956	11.0~1270	3.42
Pd(II)-FLE	276/443	ΔF=46.2+2.24×10^{3}c	0.9989	8.00~1700	2.39
Pd(II)-PEF	276/442	ΔF=20.0+4.01×10^{3}c	0.9990	6.00~1700	1.74
Pd(II)-PEF- SDBS	276/442	ΔF=284+3.56×10^{4}c	0.9979	0.43~106	0.13
Pd(II)-LEV- SDBS	293/487	ΔF=42.2+1.19×10^{4}c	0.9971	1.33~53.0	0.40
Pd(II)-LOM- SDBS	281/445	ΔF=29.3+1.17×10^{4}c	0.9963	1.66~53.0	0.50
Pd(II)-FLE- SDBS	276/443	ΔF=10.1+8.63×10^{3}c	0.9970	2.23~106	0.67

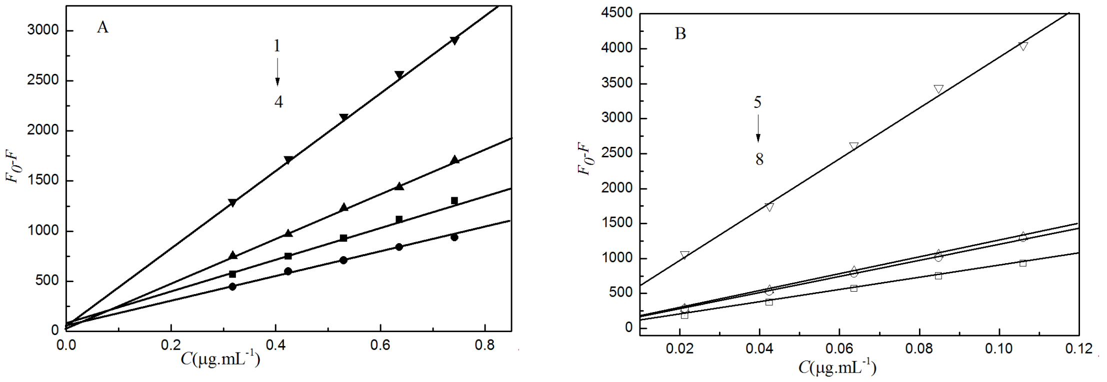


Fig. 5 The calibration graphs of Pd (II)-FLQs systems and Pd (II)-FLQs-SDBS systems

A: Pd (II)-FLQs system, PEF concentration is 3.0 μg·mL^{-1}, from curve 1 to 4 are PEF, FLE, LEV and LOM systems, B: Pd (II)-FLQs-SDBS systems, PEF concentration is 2.0 μg·mL^{-1}, SBDS concentrations are 5.0×10^{-4} mol·L^{-1}, from curve 5 to 8 are PEF, LEV, LOM and FLE systems

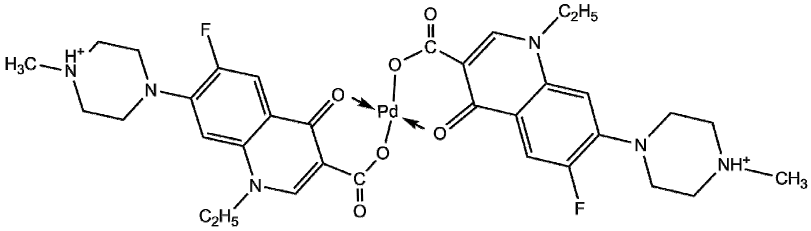
3.4 Formation of ternary complexes and synergistic quenching effect of Pd(II) and SDBS on FLQs

3.4.1 Formation of Pd(II)-FLQs and Pd(II)-FLQs-SDBS complexes

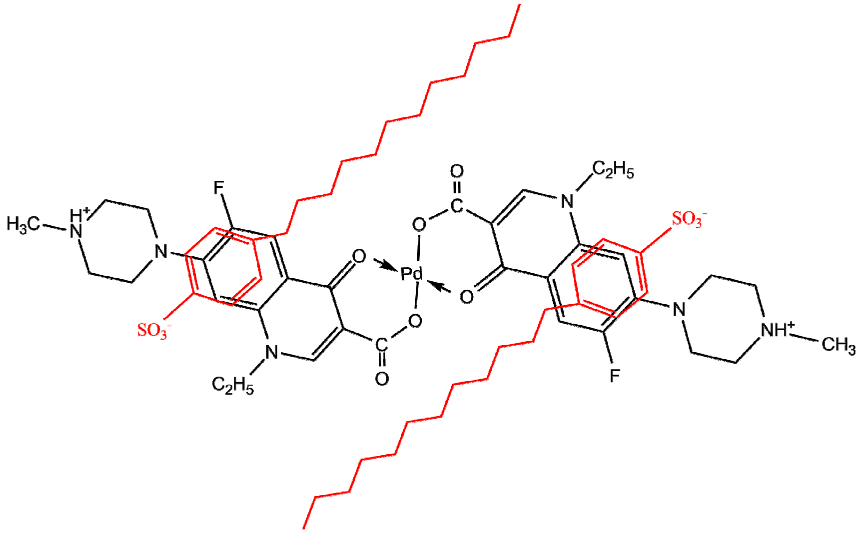
Four kinds of quinolone antibiotics have the same parent structure and fluorophore, so the mechanism of their fluorescence quenching by Pd(II) or Pd(II)-SDBS is the same. We took the most sensitive Pd(II)-PEF and Pd(II)-PEF-SDBS systems as examples to discuss the fluorescence quenching mechanism。

Under the optimum conditions, the PEF might exist as a zwitterion [33]. It was verified by the calculated results of B3LYP considering the effects of solvent. The PEF become a zwitterion ($HL^{\pm}$) due to the H^+ transferring to nitrogen atoms of piperazine ring. Namely, the molecule contain a negative —COO- and a positive heterocyclic ring nitrogen atom.

For the chelates formed by Pd(II) and PEF in 1:2 ratio, it was fully optimized by quantum chemistry in same method mentioned above. The results testified that the energy of 1:2 (Pd(II):PEF) coordination was lower than that of 1:1 coordination, indicating the 1:2 coordination was the most stable. This was consistent with the results of Job's method and molar ratio method. The results also showed that when Pd(II) reacted with PEF to form tetra-coordinated plane bidentate chelates, the energy was the lowest (-177.0 $KJ.mol^{-1}$). And the energy of the hexa-coordinated octahedron was much higher (-150.8 $KJ.mol^{-1}$). So it could be concluded that Pd(II) reacted with $HL^{\pm}$ to form 1:2 plane bidentate chelates. The structure is shown as following.



This chelate ($[Pd(HL)_2]^{2+}$) posses two positive charges which are main concentrated on the piperazine ring. Therefore, the negative charge SDBS$^-$ can bind to $[Pd(HL)_2]^{2+}$ via electrostatic attraction and hydrophobicity of long carbon chain to form ternary complexes. Our results show that, the alone SDBS react with PEF to form 1:1 ion-association complex, while in the ternary complexes the Pd(II):PEF:SDBS is 1:2:2. Although the SLS and SDS have the same structure and carbon chain as those of SDBS, they can not react with PEF and quench its fluorescence efficiently. Only SDBS containing a phenyl group can form binary and ternary complexes, therefore, it is thought that "π-π stacking interaction" between the phenyl of SDBS and aryl groups of PEF should also be an important force in addition to electrostatic attraction and hydrophobic interaction. The structure of ternary complex is shown as following.



The reaction energy and structure of Pd(II)-PEF-SDBS complex were calculated by quantum chemical calculations by way of frozen bond length. The energy difference of the combination between $[Pd(HL)_2]^{2+}$ and SDBS calculated through the formula of $\Delta E_2 = E_{Pd[HL]_2[SDBS]_2} - (E_{Pd[HL]_2^{2+}} + 2E_{SDBS^-})$, was -34.0 $kJ.mol^{-1}$. It indicated that the ternary complex was more stable than the binary complex. In the electrostatic potential diagram (Fig. 5), it can be seen that the positive charges which can bind with —SO_3^- of SDBS by electrostatic attraction are located on both sides of heterocyclic nitrogen atoms of the piperazine rings. In addition to electrostatic attraction, the π-π stacking interaction between benzene rings of SDBS and PEF play an important role. The distance between their two benzene rings is 0.46 nm.

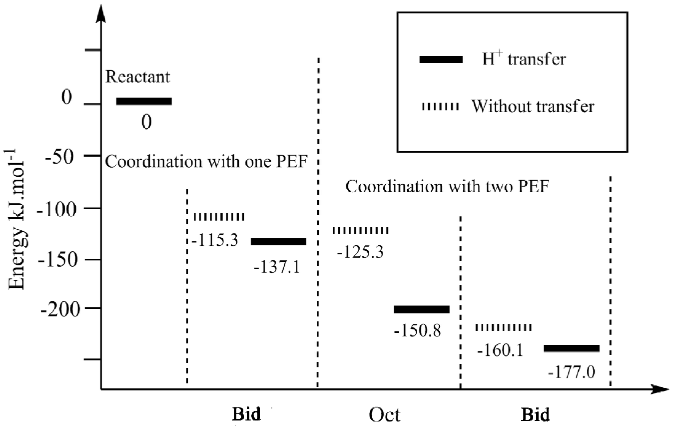


Fig. 6 Relative energy diagram of Pd(II) coordination with PEF. Bid denoted as Pd(II) coordination with PEF as plane bidentate chelates, Oct denoted as octahedron chelates

The experiments showed that the order of adding reagents did not affect the formation of ternary complexes and the fluorescence of FLQs. In experimental procedure, for determination of Pd(II), we firstly made $SDBS^-$ and $HL^{\pm}$ form 1:1 binary complex, and then added trace Pd(II) to form $[Pd(HL)_2][SDBS]_2$. According to this procedure, the reaction procedure and structure model by quantum chemical calculation were shown in Fig.7.

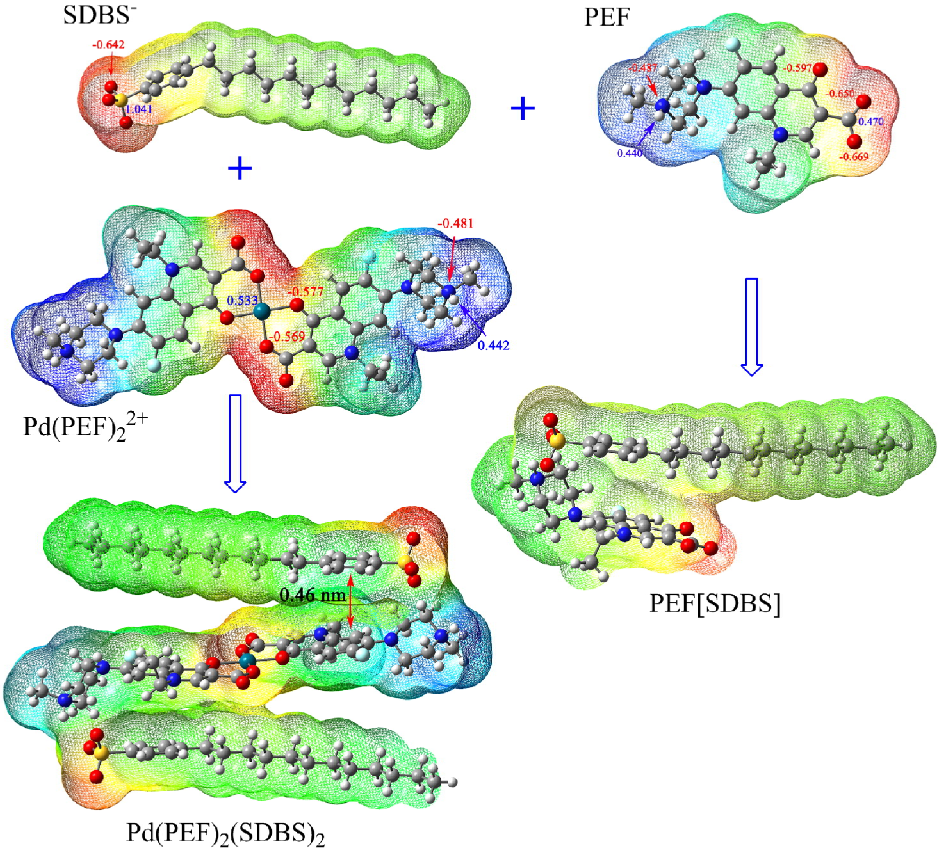


Fig. 7 Electrostatic potential diagram and selected atom charge of compounds

3.4.2 Synergic quenching fluorescence of PEF by Pd(II) and SDBS

Although alone Pd(II) or SDBS could quench the fluorescence of PEF to some different extents, they together could cause much more decrease of fluorescence of PEF. From Table 1 we can see that the quenched fluorescence intensity of PEF by certain excess of SDBS （ΔF_2） is 2157, and that by trace Pd(II) (4.0×10^{-7} mol.L^{-1}) （ΔF_1） is 190, while that by both SDBS and Pd(II) (ΔF_3) are 3887. From the phenomena of $\Delta F_1 + \Delta F_2$=2947, and $\Delta F_3>\Delta F_1+\Delta F_2$, it is thought that Pd(II) and SDBS have synergistic quenching effect on fluorescence of PEF. Similar synergistic quenching effect in color reaction of some ternary complexes and its applications in analytical chemistry are also common [34-35].

The quenching mechanism was discussed by taking Pd(II)-PEF-SDBS system as an example.

(1) Change of absorption spectra.

The absorption spectrum of PEF would change when it interacted with separate Pd(II) or SDBS, or Pd(II)-SDBS binary complex (see Fig.8). Pd(II)-PEF binary complex and Pd(II)-PEF-SDBS binary complex caused the red shifts of the maximum wavelength (λ_{max}) of PEF from 274 nm to 284 nm. And the molar absorption coeffcient decreased from 4.0×10^{-4} $L.mol^{-1}.cm^{-1}$ to 2.8×10^{-4} $L.mol^{-1}.cm^{-1}$ and 1.6×10^{-4} $L.mol^{-1}.cm^{-1}$. The absorption spectrum shape was changed, and a bigger valley appeared at 207 nm. The binding force between PEF and SDBS was weak, but there was aryl stacking interaction affecting π charges of conjugated system of PEF, so a new absorption peak appeared at 220 nm. In short, the formation of these binary and ternary complexes could cause the absorption spectral changes to some different extents.

The absorption spectral change is an important symbol of static fluorescence quenching. The absorption coeffcient of PEF decreased after it interacted with Pd(II) and SDBS. This resulted in decrease of photon transition causing fluorescence quenching. The fluorescence intensity decreased with decreasing the molar absorptivity, so the formation of low light absorption complex of Pd(II)-PEF-SDBS is an important reason of fluorescence quenching.

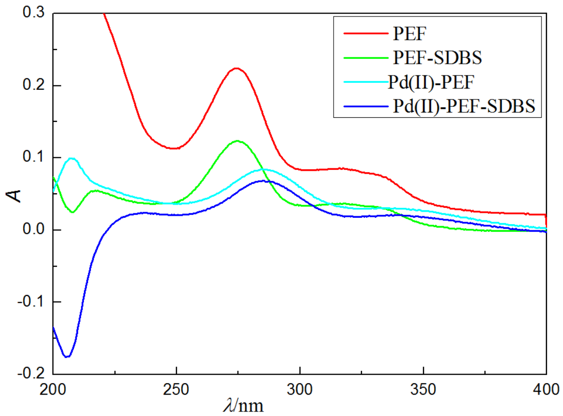


Fig. 8 Absorption spectra of Pd(II)-PEF-SDBS system. PEF concentration is 6.0×10^{-6} mol·L^{-1}, Pd(II) concentration is 1.0×10^{-5} mol·L^{-1}, SDBS concentration is 5.0×10^{-5} mol·L^{-1}, pH5.6, The absorption spectrum of PEF was recorded by using water as a reference solution. The other absorption spectra were measured using the reagent blank as the reference solution.

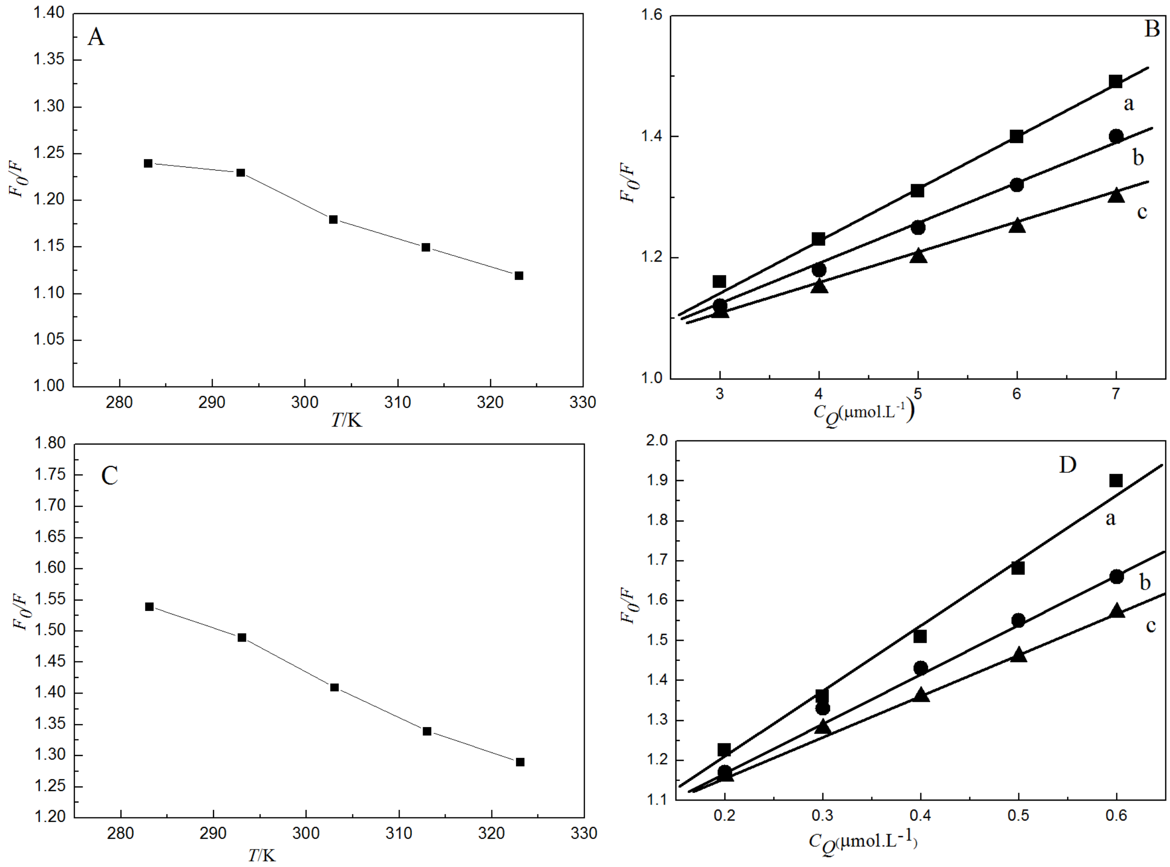


Fig. 9. Effects of temperature on fluorescence of Pd(II)-PEF and Pd(II)-PEF-SBDS systems. A：Effects of temperature on $\Delta F(F_0/F)$ of Pd(II)-PEF system. PEF concentration is 9.0 μmol·L^{-1}, Pd(II) concentration is 4.0 μmol·L^{-1}, pH5.6. B：Stem-Volmer plots of Pd(II)-PEF system at 293K(a), 303K(b) and 313K(c), Pd(II) concentrations are 3.0, 4.0, 5.0, 6.0 and 7.0 μmol·L^{-1}. C：Effects of temperature on $\Delta F(F_0/F)$ of Pd(II)-PEF-SDBS system. PEF concentration is 9.0μmol·L^{-1}, Pd(II) concentration is 0.4 μmol·L^{-1}, pH5.6. D：Stem-Volmer plots of Pd(II)-PEF-SDBS system at 293K(a), 303K(b)and 313K(c), Pd(II) concentrations are 0.2, 0.3, 0.4, 0.5 and 0.6 μmol·L^{-1}.

(2) Increase of temperature resulting in fluorescence quenching decrease

The experimental results indicated that the quenched fluorescence intensity (ΔF) of Pd(II)-PEF and Pd(II)-SDBS-PEF systems decreased with increasing temperature (Fig. 9A and C). This also illustrated the quenching effect was a single static quenching event.

3.4.3 High apparent quenching constants

For fluorescence quenching, the decrease in intensity is usually described by the well-known Stern-Volmer equation [36]:

$$F_0/F=1+K_{SV}[\mathrm{Q}] \quad (1)$$

where F_0 and F denotes the steady-state fluorescence intensities in the absence and in the presence of quencher, respectively, K_{SV} is the Stern-Volmer quenching constant, and [Q] is the concentration of the quencher. Hence, Eq. (2) was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q]. The fluorescence quenching mechanisms are usually classified as either dynamic quenching or static quenching. Dynamic and static quenching can be distinguished by their differing dependence on temperature. The quenching constants increase with the temperature increase for dynamic quenching, whereas the reverse effect is observed in case of static quenching [37]. According to the Stern-Volmer equation, Fig. 9B, 9D and Table 4 were given. For Pd(II)-PEF system, the quenching constant (K_{SV}) decreased from 8.2×10^4 L·mol^{-1} to 4.8×10^4 L·mol^{-1} with increasing temperatures from 293K to 313K. For Pd(II)-PEF-SDBS system, in the same temperature range as the above, the Ksv decreased from 1.2×10^7 L·mol^{-1} to 8.5×10^6 L·mol^{-1}. The fluorescence lifetime of PEF

(τ_0) is 10 ns [38]. The apparent quenching constants (K_q) of Pd(II)-PEF system were in the range of 4.8×10^{12} L·mol^{-1}·s^{-1}~8.2×10^{12} L·mol^{-1}·s^{-1} calculated from the equation (3):

$$K_{sv}=K_q\tau_0 \tag{2}$$

And the apparent quenching constants of Pd(II)-PEF-SDBS system were between 8.5×10^{14} L·mol^{-1}·s^{-1}and 1.2×10^{15} L·mol^{-1}·s^{-1}. They were much greater than the maximum diffusion constant ($(1.0\sim2.0)\times10^{10}$ L·mol^{-1}·s^{-1} [39]) of biomacromolecules. This also indicated the quenching effect is a static quenching event.

Table 4 Values of the quenching constants of Pd(II)-PEF and Pd(II)-PEF-SDBS systems

T/K	Pd(II)-PEF			Pd(II)-PEF- SDBS		
	K_{SV}/(L·mol^{-1})	K_q/(L·mol^{-1}·s^{-1})	R	K_{SV}/(L·mol^{-1})	K_q/(L·mol^{-1}·s^{-1})	*R*
293	8.2×10^4	8.2×10^{12}	0.9966	1.2×10^7	1.2×10^{15}	0.9996
303	7.0×10^4	7.0×10^{12}	0.9998	1.0×10^7	1.0×10^{15}	0.9998
313	4.8×10^4	4.8×10^{12}	0.9995	8.5×10^6	8.5×10^{14}	0.9999

In conclusion, based on the decreased K_{SV} values for increased temperatures, the greater K_q value than 2×10^{10} L·mol^{-1}·s^{-1} and the changed absorption spectrum, the quenching effect of Pd(II) or Pd(II)-SDBS on PEF fluorescence is a single static quenching event. The synergistic quenching effect on PEF can form to a stable ternary complex without fluorescence, thus it has higher fluorescence quenching efficiency

3.5 Selectivity and the analytical application

3.5.1 Selectivity of the method

Under the optimum experimental conditions, the effects of potentially interfering substances on the determination of Pd(II) using PEF-SDBS as a fluorescence probe were tested (see Table 5). About 470~1000 times of NO_3^-, Cl^-, PO_4^{3-}, SO_4^{2-}, 1000 times of NH_4^+, Na^+, K^+, 500 times of Ca(II) and mg(II), 300~380 times of Zn (II), Fe (III), Pb (II)， 100~290 times of Ag (I), Mn (II), Sb (III), Bi (III), Hg (II), Cd (II) and Co (II), 60 times of W (VI),10~20times of Pt (IV), Ir (III), Rh (III) and 5 times of Au (III) did not interfere with the determination. So, the method has a good selectivity.

Table 5 Effects of coexisting substances

Coexisting substance	Times	Ralative error(%)	Coexisting substance	Times	Ralative error(%)	Coexisting substance	Times	Ralative error(%)
NO_3^-	500	1.8	Mg(II)	500	3.8	Fe(III)	380	-3.2
Cl^-	500	4.1	Pb(II)	300	4.5	Al(III)	280	-4.3
SO_4^{2-}	500	2.4	Cd(II)	250	2.4	Au(III)	5	-3.9
PO_4^{3-}	470	-3.3	Zn(II)	350	2.7	Sb(III)	120	-2.8
NH_4^+	1000	-2.5	Mn(II)	120	2.5	Bi(III)	150	-3.2
Na^+	1000	1.9	Hg(II)	240	2.1	Ir(III)	20	-4.3
K^+	1000	4.3	Ni(II)	180	3.9	Rh(III)	20	-2.2
Ag^+	100	3.5	Co(II)	290	3.2	Pt(IV)	10	-3.6
Ca(II)	500	2.5	Cu(II)	25	-4.1	W(VI)	60	3.5

3.5.2 Analytical application

The concentration of Pd(II) in real samples was controlled between 4.0 and 500.0 ng·mL^{-1} by dilution or concentration. Then 1.0 mL of the treated sample was added into a 10.0 mL calibrated flask. According to the experimental procedure, 1.0 mL of BR buffer solution, 2.0 mL of 10 µg.mL^{-1} fluoroquinolone antibiotics solution, 1.0 mL of 5.0×10^{-3} mol·L^{-1} SDBS were added. The resulting solution was then diluted with water to 10.0 mL and mixed thoroughly. The concentration of Pd(II) was determined using the fluorescence quenching method (Table 6). The results were verified by those measured by ICP-AES method. The recovery is between 92.0 % and 110.0 %. The relative standard deviation was in the range of 2.8~5.6% (n=5). The determined results for Pd(II) concentration by this method and ICP-AES method were consistent.

Therefore, the method has good accuracy and precision.

Table 6 Results for the determination of Pd(II) in electroplating waste water, river water and lake water （n=5）

Sample	Found amount (μg·mL^{-1})	ICP-AES method (μg·mL^{-1})	Added amount (μg·mL^{-1})	Found total amount (μg·mL^{-1})	RSD (%)	Recovery (%)
electroplating waste water 1	190.2	187.9	200.0	401.5	3.5	105.6
electroplating waste water 2	204.3	201.9	200.0	398.9	4.1	97.3
electroplating waste water 3	8.35	8.28	10.00	18.27	2.8	99.2
electroplating waste water 4	3.52	3.01	5.00	8.31	3.7	95.8
river water	ND	ND	5.00	4.60	5.6	92.0
lake water	ND	ND	5.00	5.50	4.5	110.0

ND, not detected.

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