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Fluorescence spectroscopy determination of fluoroquinolones by charge-transfer reaction

Du Liming *, Xu Qingqin, Yuan Jianmei

Analytical and Testing Center, Shanxi Normal University, Shanxi linfen 041004, PR China

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Abstract

The charge-transfer (CT) reaction between chloranilic acid (CL) as a π-electron acceptor and lomefloxacin (LOM), fleroxacin (FLX), ciprofloxacin (CPFX), norfloxacin (NOR) as electron donor have been studied by fluorimetry. The CT complexes have stable purple color in acetone solution and the fluorescence intensity of the CT complexes was enhanced in 4–14 fold higher than that of the four fluoroquinolones (FQS) itself, therefore a new spectrofluorimetric method with simple, rapid, accurate, high sensitivity and good selectivity for determination of the four FQS has been developed. The method was applied for determination of drugs (LOM, FLX, CPFX and NOR) in tablets with mean percentage accuracies 99.80 ± 1.12, 99.93 ± 0.92, 99.23 ± 1.36 and 99.87 ± 0.81, respectively.

Keywords: Lomefloxacin; Fleroxacin; Ciprofloxacin; Norfloxacin; Sensitized fluorescence; Charge transfer complexes

1. Introduction

Fluoroquinolone (FQS) antibacterial agents are among the most important class of synthetic antibiotics. They are active against both Gram (+) and Gram (−) bacteria through inhibition of their DNA gyrase, also they have some activity against mycobacteria, mycoplasmas and rickettsias [1]. Several chromatographic methods have been reported for determination of these compounds. Lomefloxacin (LOM) [2,3], fleroxacin (FLX) [4,5], ciprofloxacin (CPFX) [6–8] and norfloxacin (NOR) [9–12] were determined by HPLC. Various spectrophotometric methods were described for determination of CPFX [13–15], NOR [16,17] and FLX [18] by charge transfer (CT) complex formation with p-benzoquinone, tetrachlorobenzoqinone, 2,3-dichloro-5,6-dicyan-p-benzo-quinohe (DDQ), chloranilic acid (CL), 7,7,8,8-tetracyanoquinodimethane (TCNQ) and 2,4-dinitrophenol. In addition, several methods have been reported for their determination such as fluorimetry [19–21] polarography [22,23], voltammetric [24,25] and capillary electrophoresis [26,27]. HPLC methods generally require tedious procedures and higher analytical costs. Fluorimetry, its main advantages over HPLC methods [2–12] are its rapidity (measurements of fluorescence are nearly instantaneous). Fluorescence spectroscopy possesses good

* Corresponding author.
E-mail address: dulim@dns.sxtu.edu.cn (D. Liming).

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analytical selectivity, higher capacity against blank interference and can improve the limit of detection when compared with spectrophotometric method [15–17]. In this paper we first reported spectrofluorimetric method for determination of LOM, FLX, CPFX and NOR through CT complexation with CL. The proposed methods are simple and suitable for routine determination of the drugs.

2. Experimental

2.1. Reagents

All solvents used were of analytical reagent grade. Methanol (Shanghai, Chemical Reagent Co., China). Acetonitrile and acetone (Tianjin Chemical Reagent Co., China). CL (Sigma Chemical Co., USA), was prepared as 3×10^{-3} mol l^{-1} in acetone. LOM, FLX, CPFX and NOR of drug standard samples were kindly provided by Chinese National Institute for the Control of Pharmaceutical and Biological Products. Stock standard solution of 100 mg l^{-1} was prepared by dissolving four-drugs standard samples in methanol as needed. Working standard were prepared by dilution of stock standard solution with acetone. Stock standard solutions was stable for several weeks at room temperature. Suppliers of drugs were as follows: LOM tablets (Searle Pharmaceutical Co., India), FLX tablets (Topefond Pharmaceutical Co., China), CPFX tablets (Xinchang Pharmaceutical Co., China) and NOR capsules (Taiyuan Pharmaceutical Co., China).

2.2. Apparatus

Fluorescence signals were measured on a LS-50B luminescence spectrometer (Perkin–Elmer, USA) equipped with a xenon-lamp and computer working with the LS-50B software. All the measurements took place in a standard 10 mm pathlength quartz cell, thermostated at 25.0 ± 0.5 °C, with 2.5 nm bandwidths for the emission and excitation monochromators. A SHIMDZU UV-2201 ultraviolet–visible spectrophotometer (Tokyo, Japan) was used for the absorbance measurements.

2.3. Procedure

2.3.1. General procedure

A suitable amount of drug solution was pipetted into a 10-ml volumetric flask, 1.0 ml of CL solution was added, and the solution was diluted to volume with acetone and mixed thoroughly. The flasks with solution were placed for 30 min for LOM and 20 min for CPFX in 35 °C water bath, and 30 min in 30 °C water bath both for FLX and NOR, respectively. The complexes of LOM and CPFX were measured at 438 and 427 nm using an excitation wavelength of 335 and 333 nm against a blank solution, respectively. FLX and NOR were measured at 436 and 431 nm using an excitation wavelength of 334 and 335 nm against a blank solution, respectively. The concentration of LOM, FLX, CPFX and NOR in the sample were determined from a calibration graph prepared under identical condition.

2.3.2. Procedure of tablets of lomefloxacin, fleroxacin and ciprofloxacin

Ten tablets of drugs were weighed and pulverized carefully, certain amount of powder (containing about 100 mg of LOM or FLX or CPFX) was dissolved well and diluted to mark of 100 ml calibrated flask with acetone. The solution was filtered, the first 10 ml of the filtrate was discarded, the 10 ml of continuation of sample solution was diluted to ten times volume with acetone and was tested as under Section 2.3.1.

2.3.3. Procedure for capsules of norfloxacin

Ten capsules of NOR were weighed and pulverized; certain amount of powder (containing about 100 mg of NOR) was dissolved well and diluted to mark of 100 ml calibrated flask with acetone. The sample solution was filtered and tested as described above.

3. Results and discussion

3.1. Excitation spectra and emission spectra

CL was found to react with some drugs to produce purple color products—CT complexes

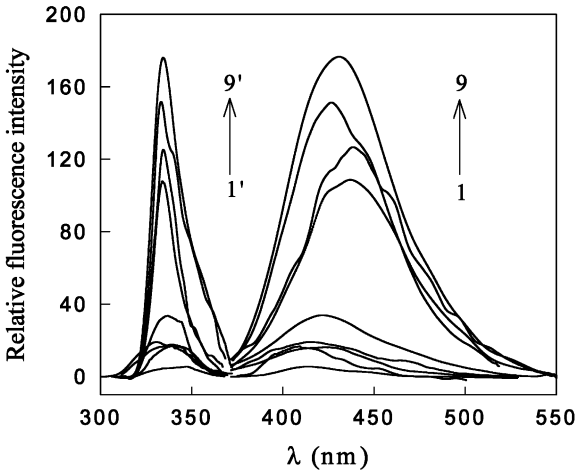

Fig. 1. Fluorescence spectra of: (1, 1′) CL (3×10^{-4} mol l^{-1}). (2, 2′) CPFX (1.06 μg ml^{-1}). (3, 3′) NOR (1.02 μg ml^{-1}). (4, 4′) FLX (1.18 μg ml^{-1}). (5, 5′) LOM (1.12 μg ml^{-1}). (6, 6′) FLX (1.18 μg ml^{-1})–CL. (7, 7′) LOM (1.12 μg ml^{-1})–CL (8, 8′) CPFX (1.06 μg ml^{-1})–CL. (9, 9′) NOR (1.02 μg ml^{-1})–CL. (1′–9′) Excitation spectra. (1–9) Emission spectra.

3.3. Effect of solvent

The studied solvents involved water, methanol, ethanol, isopropanol, acetone, acetonitrile and chloroform. Experimental results indicated that acetone gave the maximum and stable emission for 4-FQS. Other solvents as methanol or ethanol were unsuitable due to limited solubility of the concerned drugs. Chloroform was unsuitable as CL have limited solubility in it.

3.4. Mechanism of reaction

CL is an π-acceptor, LOM, FLX, CPFX and NOR are nitrogenous compounds. So CL complexes can be formed with these drugs. Molar ration of the reactants in the CT complex was determined by Bent–French and curved intersection method and it was found to be 1:1 for 4-FQS with CL. This ratio may be due to the presence of the fluorine atom acting as an electron drawing

Table 1
Reaction temperature and time for CL with 4-FQS

Drugs	Lomefloxacin	Fleroxacin	Ciprofloxacin	Norfloxacin
Reaction temperature (°C)	35	30	35	30
Reaction time (min)	30	30	20	30
Stable time (min)	60	80	60	70

with the changes of fluorescence quantum efficiency, resulting in an important improvement in fluorescence intensity. As shown in Fig. 1, four FQS can emit only very weak fluorescence in acetone, while the fluorescence intensity of solution after reaction with CL was enhanced in 4–14 fold higher than that of FQS itself.

3.2. Effect of reaction time and temperature

Effect of reaction time on fluorescence intensity of FQS solution was tested at various temperature (20, 25, 30, 35, 40, 45 and 50 °C), respectively. The Table 1 listed the experimental results about the suitable temperature and time for obtaining maximum, stable fluorescence emission spectra and the stable time of CL complex at room temperature.

group in the molecule of 4-FQS. The benzene ring has lower electron density, but nitrogen atom in four of piperazingl has more electron density and less sterically hindered. So n-π CT complexes were formed (Fig. 2).

3.5. Calibration graph and sensitivity

Under the experimental conditions described, standard calibration curves for LOM, FLX, CPFX and NOR with CL were constructed by plotting fluorescence intensity versus concentration, the linear regression equation for each method are listed in Table 2. The correlation coefficients were 0.9991–0.9998, indicating good linearity. The small value of variance confirmed

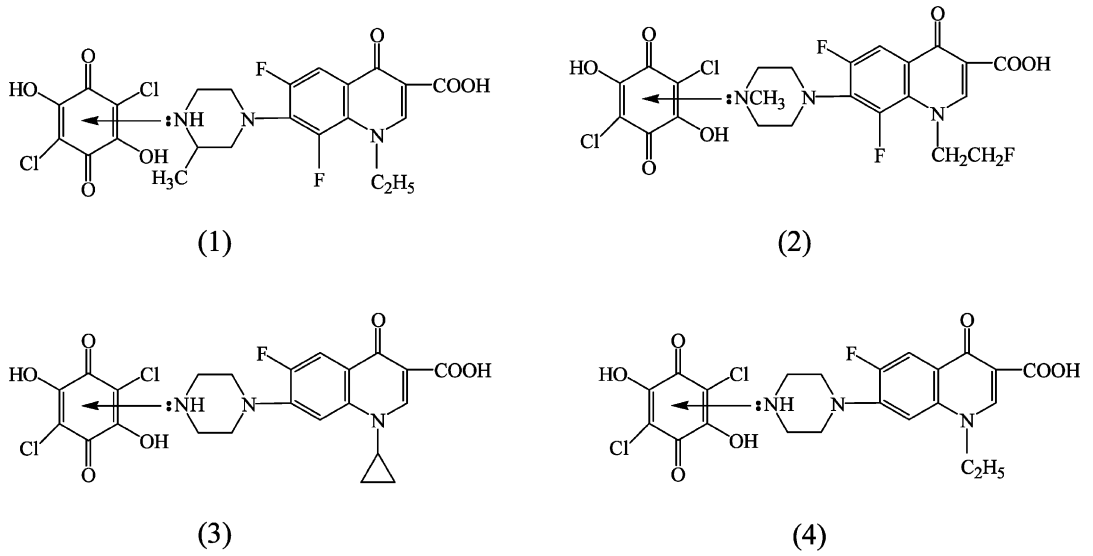

Fig. 2. The structure of 4-FQS–CL (CT) complexes. (1) LOM–CL (CT) complex. (2) FLX–CL (CT) complex. (3) CPFX–CL (CT) (CT) complex. (4) NOR–CL (CT) complex.

Table 2
Characteristic parameters for complexes of LOM, FLX, CPFX and NOR with CL

Parameters	LOM	FLX	CPFX	NOR
$\lambda_{ex}/\lambda_{em}$ (nm)	335/438	334/436	332/427	334/431
Linear range (μg ml^{-1})	0.14–8.2	0.16–11.4	0.10–7.2	0.08–5.6
Limit of detection (μg ml^{-1})	0.08	0.09	0.06	0.02
Limit of quantitation (μg ml^{-1})	0.14	0.16	0.10	0.08
Slope (b)	113.3	98.22	141.51	178.52
S.D. of Slope (S_b)	0.91	1.17	0.85	2.49
Intercept on the ordinate (a)	1.314	0.462	− 1.198	2.231
S.D. of the intercept on the ordinate (S_a)	0.17	0.06	0.02	0.03
Variance (S_0^2)	0.112	0.024	0.002	0.008
Number of points (n)	25	27	32	16
Correlation coefficients (r)	0.9995	0.9998	0.9991	0.9992

the small degree of scattering of the experimental data points around the regression line.

3.6. Analysis of pharmaceutical formulations

The proposed methods were applied to the determination of LOM, FLX and CPFX in commercial tablets and NOR in capsules. Five replicate determinations were made. Satisfactory results were obtained for four drugs (Table 3). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding LOM, FLX, CPFX and NOR to the previously analyzed tablets. The recovery of each drug was calculated by comparing the concentration obtained from the (spiked) mixtures with those of the pure drugs. Table 3 shown the results of analysis of the commercial tablets,

Table 3
Determination of drugs in pharmaceutical formulation using CL ($n = 5$)

Drugs	Present method			Reference method	
	Found (mg per grain)	Equivalent nominal content ± S.D. (%)	Recovery (%)	Found (mg per grain)	Equivalent nominal content (%)
Lomefloxacin table	396.2	99.13 ± 1.52 (t, 1.28; F, 3.34)	99.80 ± 1.22	395.4	98.92 ± 0.83 [19]
Fleroxacin, table	99.2	99.21 ± 1.36 (t, 1.30; F, 2.34)	99.93 ± 0.92	99.6	99.63 ± 0.89 [18]
Ciprofloxacin table	248.9	99.64 ± 0.9 (t, 0.83; F, 1.24)	99.23 ± 1.36	249.2	99.70 ± 0.87 [28]
Norfloxacin capsule	98.2	99.23 ± 1.38 (t, 0.27; F, 2.07)	99.17 ± 0.81	98.7	99.74 ± 0.96 [28]

The tabulated values of t and F at the 95% confidence limit are $t = 2.78$ and $F = 6.39$.

Table 4
Precision results of FQS ($n = 11$)

Concentration (μg ml^{-1})	Within-day R.S.D. (%)				Between-day R.S.D. (%)			
	LOM	FLX	CPFX	NOR	LOM	FLX	CPFX	NOR
0.10	1.2	1.1	1.2	1.4	1.4	1.2	1.4	1.6
1.00	1.6	1.4	0.9	1.3	1.5	1.4	1.2	1.4
6.00	1.3	1.2	0.7	1.1	1.4	1.3	0.9	1.3

capsule and the recovery study (standard addition method) of four drugs. Comparison of the results obtained by the proposed method with those obtained by official method [28] and literature method [18,19]. The accuracy is satisfactory.

The obtained high-intensity fluorescence bands and the very low reagent background make these procedures suitable for the routine quality control analysis of the investigated compounds with minimum interference. The proposed and reference methods were applied to the determination of the studied drugs in tablets and capsules containing different FQS (Table 3). The obtained mean values (±S.D.) of the labeled amounts ranged from 99.13 ± 1.52 to 99.64 ± 1.02. In the t-and F-tests, no significant differences were found between the calculated and theoretical values (95% confidence) of both the proposed and reference methods. This indicates similar precision and accuracy.

3.7. Precision

Precision of the proposed methods was determined in each concentration range, by 11 measurements carried out on different days within a week of different solution of LOM, FLX, CPFX and NOR. Target concentrations corresponded to middle values in each range. Table 4 gives a R.S.D. (within-day and between-day) of solutions of 0.10, 1.00 and 6.00 μg ml^{-1} were determined by using the proposed procedure.

3.8. Effect of interfering substances

A study of some potential interfering substances in the spectrofluorimetric determination of 4-FQS was performed by selecting them as the excipients often used in table formulations. Samples containing a fixed amount of the FQS (1.0 μg ml^{-1}) and variable concentrations of excipients were measured. Lactose, sucrose, glucose and fructose do not cause interference at weight ratios of excipient/the FQS $< 10\,000$.

4. Conclusion

The results obtained from the present study indicate that complex formation between the FQS and CL be employed in the spectrofluorimetric

assay of LOM, FLX, CPFX and NOR in its dosage forms. The proposed methods are suitability for the routine quality control of the drug alone and in tablets or capsules without fear of interference caused by the excipients expected to be present in tablets or capsules. The method has been also applied successfully to the determination of the active constituent in a commercial pharmaceutical.

Acknowledgements

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