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Study of fluorescence characteristics of the charge-transfer reaction of quinolone agents with bromanil

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ABSTRACT

A spectrofluorimetric method was discussed for the determination of three antibacterial quinolone derivatives, ofloxacin (OFL), norfloxacin (NOR) and ciprofloxacin (CIP) through charge-transfer complexation (CTC) with 2,3,5,6-tetrabromo-1,4-benzoquinone (bromanil, TBBQ). The method was based on the reaction of these drugs as n-electron donors with the π-acceptor TBBQ. TBBQ was found to react with these drugs to produce a kind of yellow complexes and the fluorescence intensities of the complexes were enhanced by 29–36 times more than those of the corresponding monomers. UV–vis, ^{1}H NMR and XPS techniques were used to study the complexes formed. The various experimental parameters affecting the fluorescence intensity were studied and optimized. Under optimal reaction conditions, the rectilinear calibration graphs were obtained in the concentration range of 0.021–2.42 μg mL^{-1}, 0.017–2.63 μg mL^{-1} and 0.019–2.14 μg mL^{-1} for OFL, NOR and CIP, respectively. The methods developed were applied successfully to the determination of the subject drugs in their pharmaceutical dosage forms with good precision and accuracy compared to official and reported methods as revealed by *t*- and *F*-tests.

1. Introduction

Ofloxacin (OFL), norfloxacin (NOR) and ciprofloxacin (CIP) are the three representative drugs of the third generation quinolone antibiotics agents. The structure of the drugs was shown in Fig. 1. Fluoroquinolones evolved rapidly into drugs of a full synthetic anti-jamming kind, and gained extensive clinical application within a few decades. They have many advantages, including a broad antibacterial spectrum, good oral absorption, and high concentrations of drugs in human plasma. The dissociative salts or shaped salts of these drugs can be made into various pharmaceuticals that induce fast distribution in body tissue and a longer half-life. They are not only effective against most Gram-negative and Gram-positive aerobic bacteria, but also highly active in suppressing mycobacteria, mycoplasmas, rickettsias and the protozoan plasmodium falciparm. Due to their extensive clinical application, more and more analysis methods were reported, which received close attention in analytical chemistry. For quality control purpose, methods such as the following were developed: spectrophotometry [1–3], thin-layer chromatogram [4,5], high performance liquid chromatography [6], flow injection chemiluminescence [7,8], high performance capillary electrophoresis [9,10], polarography [11,12], etc. Though these methods are sensitive enough, they are expensive and not easily manageable. On the other hand, the determination of quinolone agents based on charge-transfer reaction is limited to spectrophotometry [13–17].

An extensive study has been carried out on the possible role or CT and inner (σ) complexes as reaction intermediateds in the reaction of organic and inorganic molecules with electron acceptors, particularly quinines [17,18]. However, to the best of our knowledge, the mechanism of CT reaction and how to prove the CTC formation by XPS and NMR etc are seldom in the literature. Based on careful study on benzoquinone–acceptor and quinolone agents–donor we found that OFL, NOR and CIP reacted very easily with 2,3,5,6-tetrabromo-1,4-benzoquinone (bromanil, TBBQ) under appropriate conditions, resulting in kinds of yellow charge-transfer complex compounds. Additionally, the fluorescence emission intensified significantly. Based on this phenomenon, we determined the amount of OEL, NOR and CIP in commercial pharmaceuticals, which proved to be satisfactory. In this paper, the mechanism of charge-transfer reaction was studied and ^{1}H NMR and XPS techniques were applied to investigate the charge-transfer complexes. The proposed method is simple, high sensitive, of good analytical selectivity and suitable for routine determination of the drugs.

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2. Materials and methods

2.1. Apparatus

Fluorescence signals were measured on a Cary Eclipse fluorescence spectrophotometer (VARIAN, USA) equipped with a xenon lamp. All the measurements took place in a standard 10 mm pathlength quartz cell, with 2.5 nm bandwidths for the emission and excitation monochromators.

A Lambda Bio 40 UV–vis spectrophotometer (PerkinElmer, USA) was used for the absorbance measurements.

The ^{1}H NMR spectrometer was a Bruker DRX300, 300.13 MHz (Bruker, Switzerland), used for the changes of ^{1}H spectrum.

An Escalab 250 X-ray photoelectron spectroscopy (VG, UK) was used for recording the binding energy spectrum.

2.2. Reagents

All solvents used were of analytical reagent grade. Suppliers were as follows: acetone and acetonitrile (Nanjing Chemical Reagent Co., Ltd., China), DMSO and d_6-DMSO (Beijing Chemical Plant, China), methanol (Nanjing Chemical Reagent No.1 Plant, China), ethanol (Tianda Chemical Reagent Plant, Tianjin, China), chloroform (Kemiou Chemical Reagent Research Center, Tianjin, China), water used in all experiment was redistillation product.

TBBQ (Aldrich Chemical Co., USA) was prepared as 1.0×10^{-3} mol/l in methanol, solution was found to be stable for at least 1 week at room temperature. OFL, NOR and CIP of drug standard samples were kindly provided by Shanxi Provincial Institute for Drug Control. A standard stock solution of 100 μg mL^{-1} was prepared by dissolving three drugs standard samples in methanol as needed. A standard working solution was prepared by diluting a standard stock solution with methanol. The standard stock solution was stable even after several weeks at room temperature.

2.3. Pharmaceutical formulation

The following commercial dosage forms were subjected to the analytical procedure: Ofloxacin tablets (Lizhu Pharmaceutical Co., Ltd., Zhuhai, China), labeled as containing 100 mg ofloxacin per tablet; Norfloxacin capsules (Shanxi Haisi Pharmaceutical Co., Ltd., Jincheng, China), labeled as containing 100 mg norfloxacin per grain. Ciprofloxacin hydrochloride tablets (Tianfang Pharmaceutical Co., Ltd., Henan, China), labeled as containing 250 mg ciprofloxacin per tablet.

2.4. Procedures

2.4.1. General analytical procedures

A suitable amount of OFL, NOR and CIP standard stock solutions were transferred to three 10 mL calibrated flasks. 0.5 ml, 0.6 ml and 0.4 ml of TBBQ solution was added for OFL, NOR and CIP, respectively, and each solution was diluted to 10 ml with methanol (for OFL and NOR) and with the mixed solvent (volume ration of methanol to ethanol is 4:6, for CIP) and thoroughly mixed. The solutions were placed in a 50.0 ± 0.5 °C (for OFL and NOR) and 40.0 ± 0.5 °C (for CIP) water bath for 30 min. Then the flasks were cooled to room temperature and the fluorescence intensities of the charge-transfer complexes of OFL, NOR and CIP were measured at 487 nm, 437 nm and 435 nm using an excitation wavelength of 286 nm, 274 nm and 276 nm against a blank solution, respectively. The calibration graph was constructed in the same way with solutions of the subject drugs of known concentrations. The amount of drugs in the sample solutions was determined from their calibration graphs.

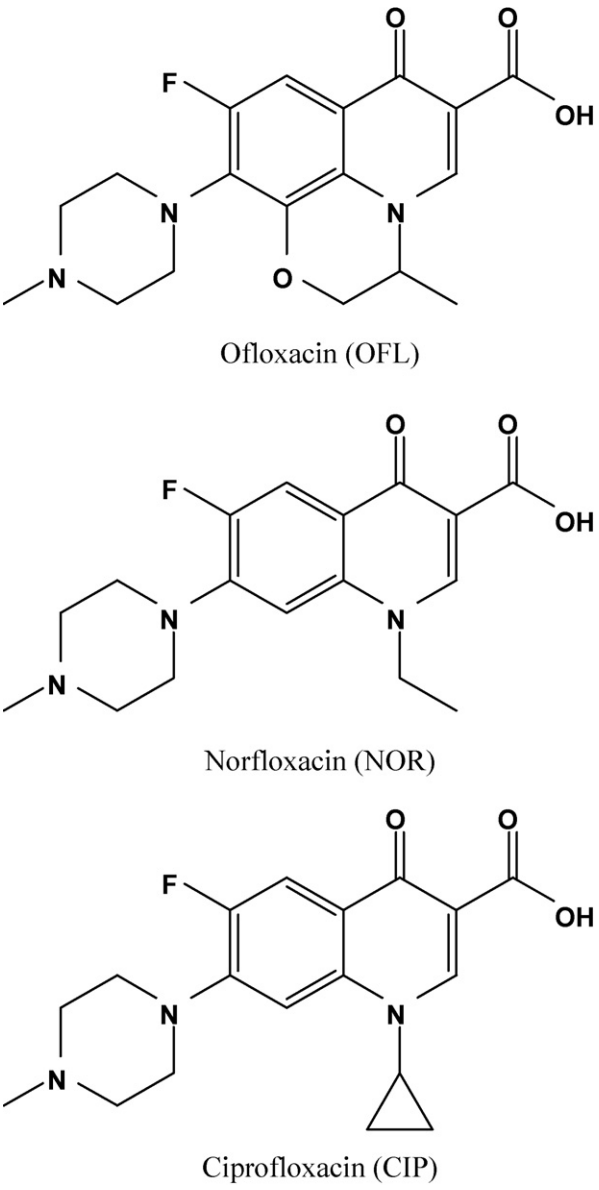


Fig. 1. The molecular structure of quinolone antibacterial agents.

2.4.2. Analysis of tablets and capsules

Ten tablets or capsules of each drug were weighed and powdered or evacuated. An amount of the powdered tablet equivalent to 100 mg of each drug was weighed accurately and transferred into a 100 ml calibrated flask, dissolved in 2 ml of methanol, swirled and sonicated for 2 min, the solution was diluted to volume with methanol. The first 10 ml of the filtrate was discarded, and the next 10 ml of sample solution was diluted to 100 times volume with methanol. After the solution was adequately mixed, the procedure described in Section 2.4.1 was followed.

2.4.3. Solutions for ^{1}H NMR measurements

7.22 mg, 6.38 mg and 7.72 mg of OFL, NOR and CIP were accurately weighed and were dissolved in 0.5 ml d_6-DMSO, respectively,

and 0.5 ml solution containing an equimolar amount of TBBQ in the same solvent was added and subjected directly to ^{1}H NMR measurements.

2.4.4. Preparation of the complexes for XPS measurements

10 ml of 0.002 M of each drug in methanol, as well as 10 ml of 0.002 M of TBBQ in methanol, was added to three round-bottom flasks separately and stirred for 30 min. The solvent was evaporated under reduced pressure and the resulting residues were dried over calcium chloride for further examination.

3. Results and discussion

3.1. Excitation spectra and emission spectra

Fig. 2a shows the excitation and emission spectra for the subject drugs and its CT complexes with TBBQ. It can be seen that the solutions of the three drugs have weak native fluorescence and the emission peaks of OFL, NOR and CIP are at 475 nm, 415 nm and 421 nm, respectively. However, in the presence of TBBQ, the fluorescence intensity increases substantially, and the sensitivity is enhanced by 29–36 times, with red shifts to 487 nm, 437 nm and 435 nm, respectively. The modification of the features of the fluorescence spectra was considered to be a result of formation of CT complexes between the subject drugs and TBBQ. (In order to describe 4–7 and 4′–7′ in Fig. 2a clearly, their spectra were shown in Fig. 2b.)

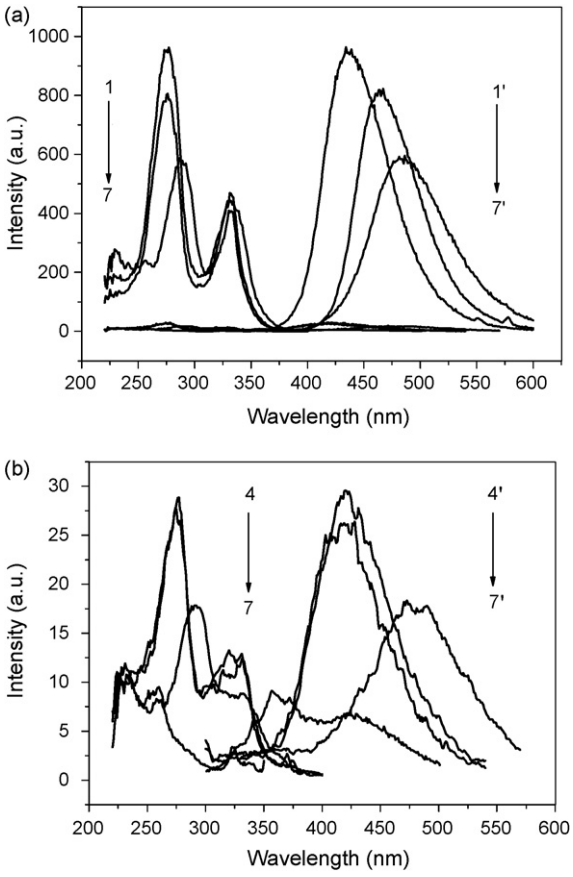


Fig. 2. Fluorescence excitation and emission spectra.

3.2. Solvent selection

Several solvents such as methanol, ethanol, acetone, chloroform, acetonitrile, DMSO, redistillation water were evaluated. The fluorescence intensities of CT complexes of OFL, NOR and CIP in different solvents are shown in Fig. 3. Experimental results indicated that methanol was an ideal solvent for OFL and NOR, as it gave the maximum and most stable fluorescence emission, and that a mixed solvent of methanol–ethanol was suitable for CIP, the reaction system gaining maximum fluorescence intensity and sensitivity (see Fig. 4) at a ratio of methanol to ethanol being 4:6.

3.3. Effect of reaction temperature and time

The effect of temperature on the CT complexes formed was studied in the range of 20–60 °C. The results obtained indicate that the fluorescence intensity is enhanced as the temperature increases. However, it was found that the effect of temperature became negligible after 50 °C for OFL and NOR, 40 °C for CIP. Therefore, the examination of the subject drugs were carried out at 50 ± 0.5 °C

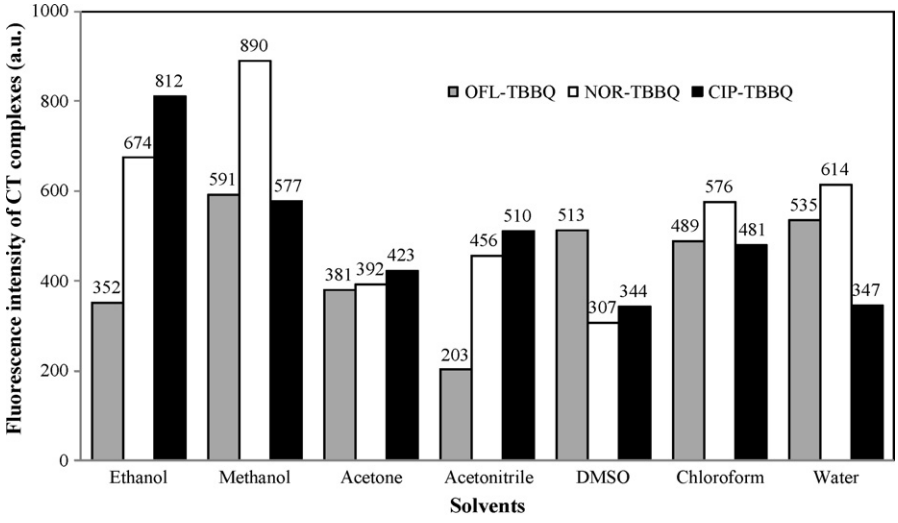


Fig. 3. Fluorescence intensity of charge-transfer complexes in solvents.

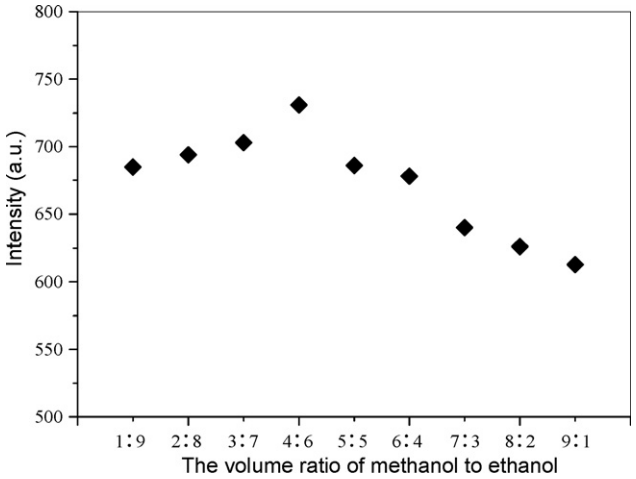


Fig. 4. Effect of the volume ratio of methanol to ethanol on the charge-transfer reaction of CIP–TBBQ.

for OFL and NOR, and at 40 ± 0.5 °C for CIP. It was further found that the complexes completely formed after 30 min, after which they remained stable for at least 2 h.

3.4. Effect of TBBQ concentration

The effect of the amount of TBBQ on the CT complexes of the subject drugs was investigated. Our work indicates that 0.5 ml, 0.6 ml and 0.4 ml of TBBQ solution were optimal for OFL, NOR and CIP, respectively (see Fig. 5). As the TBBQ concentration was lower than optimum value, the charge-transfer reaction was not complete. Furthermore, the fluorescence intensity decreased, resulting from the increased colliding probability of the reactant molecules when the concentration is higher.

3.5. Effect of interfering substances

A study of potential interference of some substances (namely, the frequently used excipients or additives in pharmaceutical formulations) with the spectrofluorimetric examination of the subject drugs was performed under optimized conditions. Samples containing a fixed amount of the subject drugs (2.0 μg mL^{-1}) and variable concentrations of excipients were measured. The result showed that the coexistent organic compounds, such as 1000 times lactose, sucrose and starch, 500 times glucose, 200 times magnesium stearare did not induce significant interference in the examination. This fact indicates good selectivity of the method to determine the subject drugs in both raw and dosage form.

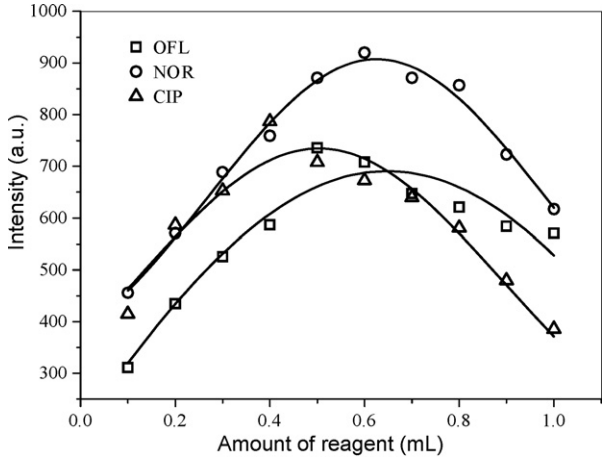


Fig. 5. Effect of the amount of TBBQ on fluorescence intensity of complexes.

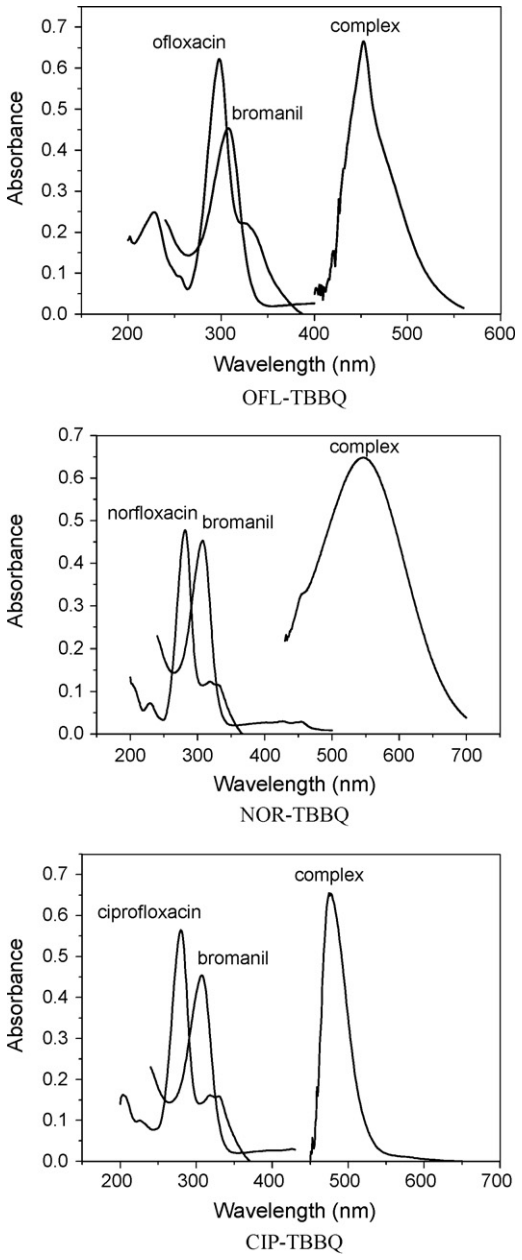
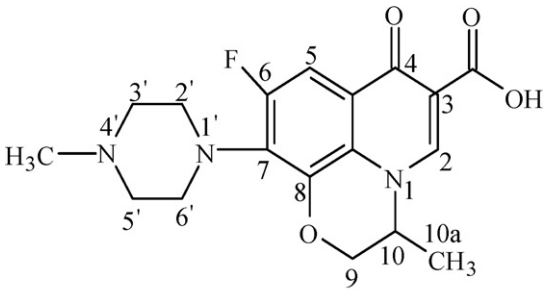


Fig. 6. UV–vis spectra of OFL–TBBQ, NOR–TBBQ and CIP–TBBQ.

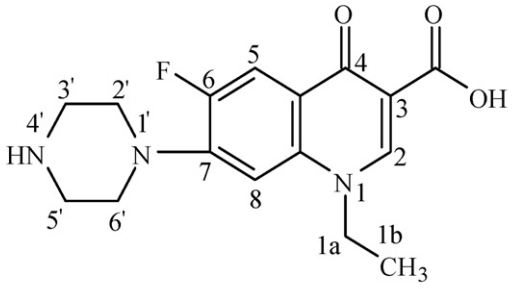
3.6. Research on the mechanism of reaction

3.6.1. Characteristics of CT complexes by UV–vis

The absorption spectra of the subject drugs and TBBQ reagent as well as their complexes are shown in Fig. 6. It shows a characteristic maxima at λ_{max} = 450, 550 and 475 nm for OFL-TBBQ, NOR-TBBQ and CIP-TBBQ CT complexes, respectively, while the maximum absorption wavelengths of individual TBBQ, OFL, NOR and CIP were 308, 298, 282 and 280 nm, respectively. Thus, the obvious red shifts indicate charge-transfer complexation between TBBQ and the subject drugs.



OFL



NOR

Fig. 7. The molecular structure of ofloxacin and norfloxacin.

3.6.2. *Characterization of CT complexes by ^{1}H NMR [19,20]*

Comparison between the chemical shifts in the ^{1}H NMR spectra before and after reaction helped in ascertaining the exact binding site of OFL and NOR. In order to describe clearly, we labelled the position in the molecular structure of OFL and NOR, as shown in Fig. 7. The ^{1}H NMR spectra of the complexes of the subject drugs and the corresponding monomers were recorded, and the chemical shifts are list in Table 1.

In the ^{1}H NMR spectra of the complexes of OFL and NOR, since only 4′-CH_3, 3′,5′-CH_2 ($\Delta\delta$ = 0.109, 0.150 ppm) in OFL and 3′,5′-CH_2, 2′,6′-CH_2 ($\Delta\delta$ = 0.651, 0.470 ppm) in NOR are shifted downfield, other protons of the complexes showed no evident changes. This could be explained as follows: the unpaired electron on the 4′-N transferred to TBBQ, which induced a decrease of electron cloud density on the proton of 4′-CH_3 and piperazinyl-CH_2, as a result, a downfield chemical shift occurred. This indicated that the complexation binding of TBBQ with the 4′-N on the piperazinyl of NOR and OFL gave rise to the CT complexes.

3.6.3. *The characterization of CT complexes by XPS [21]*

XPS determination was carried out on the Escalab250 XPS (VG, the UK). The binding energy changes occurring on OFL, NOR and CIP before and after their complexation with TBBQ were observed, and the charge-transfer information obtained was helpful in ascertaining the specific complexation site. The XPS spectra were shown in Fig. 8. For illustrative purpose, the binding energy data were included into Table 2. As in the table, the N_{1s} binding energy of OFL, NOR and CIP increases from 399.33 eV, 399.45 eV and 399.80 eV to 399.80 eV, 399.72 eV and 400.10 eV after complexation, respectively. In other words, increases of 0.47 eV, 0.27 eV and 0.30 eV were observed for OFL, NOR and CIP, respectively. On the other hand, the Br_{3d} binding energy of TBBQ decreased from 70.65 eV to 69.47 eV, 70.04 eV and 69.79 eV for OFL, NOR and CIP, respectively, after complexation. The corresponding binding energy reductions were 1.18 eV, 0.61 eV and 0.86 eV, respectively. Again, this could be explained by the electron transfer that occurred during complexation. Specifically, the electron on N_{1s} of OFL, NOR and CIP transferred to TBBQ during the formation of CT complexes, which resulted in an electron density decreases around the N_{1s} proton of OFL, NOR and CIP, giving rise to a binding energy increase. On the other hand, the transfer of electron onto TBBQ caused an electron density increase of Br proton on TBBQ, which resulted in a binding energy decrease. This indicates that the complexation occurred between the Nitrogen of the quinolones and TBBQ, which corroborated the inference from the NMR experiments.

According to CT theory and the characterization of CT complexes with UV–vis, ^{1}H NMR and XPS, we found that the TBBQ molecule

Table 1
Chemical shifts data of ^{1}H NMR of ofloxacin, norfloxacin and its complex (δ/ppm)

Compounds	OFL	OFL–TBBQ	$\Delta\delta$	NOR	NOR–TBBQ	$\Delta\delta$
2-CH	8.943(s[a])	8.932(s)	0.011	8.913(s)	8.951(s)	0.038
5-CH	7.539(d[b])	7.535(d)	0.004	7.853(d)	7.937(d)	0.084
8-CH	–	–	–	7.107(d)	7.205(s)	0.098
1a-CH_2	–	–	–	4.553(d)	4.577(s)	0.024
1b-CH_3	–	–	–	1.376(s)	1.386(s)	0.010
9-CH_2	4.442(m[c])	4.429(m)	0.013	–	–	–
10-CH	4.898(s)	4.875(s)	0.023	–	–	–
10a-CH_3	1.418(s)	1.406(s)	0.012	–	–	–
4′- CH_3	2.198(s)	2.307(s)	0.109	–	–	–
3′,5′-CH_2	2.405(s)	2.555(s)	0.150	2.858(s)	3.509(s)	0.651
2′,6′-CH_2	3.265(s)	3.292(s)	0.027	3.190(s)	3.660(s)	0.470

[a] The representation of single peak.
[b] The representation of double peak.
[c] The representation of multi peaks.

Table 2
Changes of binding energy of OFL, NOR, CIP and TBBQ in charge-transfer reactions

Binding energy (eV)	N_{1s}			Br_{3d}			
	OFL	NOR	CIP	TBBQ	OFL	NOR	CIP
Before reaction	399.33	399.45	399.80	70.65	–	–	–
After reaction	399.80	399.72	400.10	–	69.47	70.04	69.79
Difference value	0.47	0.27	0.30	–	−1.18	−0.61	−0.86

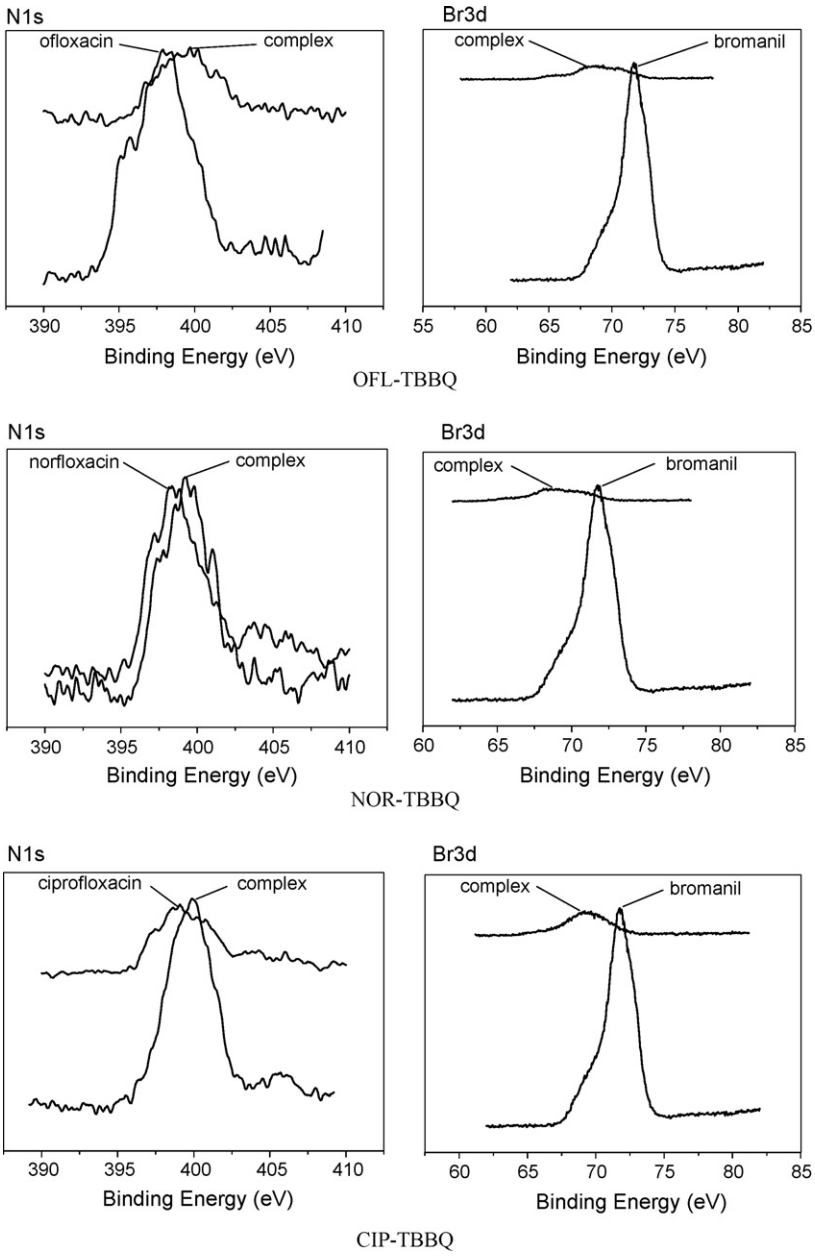


Fig. 8. XPS spectra of N_{1s} and Br_{3d} in three charge-transfer complex compounds.

is a plane π-electron acceptor with a low electron cloud density on the benzene ring. The OFL, NOR and CIP were N containing compounds. The single paired electrons on the 4′N of piperazinyl as well as a low steric hindrance made them good electron donors. Under suitable conditions, TBBQ could form n–π CT complexes with OFL, NOR and CIP. The molar ration was 1:1 for all three CT complexes as revealed by molar ratio method and Bent–French method. The probable patterns of CT complexes were shown in Fig. 9.

3.7. The calibration curve

A series of standard solutions were prepared with known concentration, and then subjected to the procedures under optimal experimental conditions. The reaction products were evaluated on the fluorescence spectrophotometer. Then the fluorescence intensity was plotted against the concentration to give the calibration curve, the linear range and the regression equation, as shown in Table 3. The linear coefficient for various solutions was in the range 0.9995–0.9998, which indicates good linearity. We also calculated the detection limit and quantification limit in this paper according to the IUPAC.

Table 3
Characteristic parameters for complexes of three quinolone with TBBQ

Parameters	OFL-TBBQ	NOR-TBBQ	CIP-TBBQ
$\lambda_{ex}/\lambda_{em}$ (nm)	286/487	274/437	276/435
Linear range ($\mu g\,mL^{-1}$)	0.021–2.42	0.017–2.63	0.019–2.14
Slope	322.50	349.41	395.48
Intercept	5.81	2.92	4.04
Correlation coefficients (r)	0.9997	0.9998	0.9995
Standard deviation (s_0)	0.693	0.581	0.756
Limit of detection ($\mu g\,mL^{-1}$)	0.0064	0.0050	0.0057
Limit of quantification ($\mu g\,mL^{-1}$)	0.021	0.017	0.019

Table 4
Results for the examination of drugs in pharmaceutical formulations ($n = 7$)

Drugs	Present method[a]				Reference method	
	Found (mg/grain)	Equivalent nominal content (%)	Recovery (%)	R.S.D. (%)	Found (mg/grain)	Equivalent nominal content (%)
OFL tablets	99.2	99.24 ± 1.21; $t = 1.30$, $F = 2.37$	99.91 ± 1.32	2.4	98.1 [1]	98.12 ± 0.98
NOR capsules	98.5	98.48 ± 1.48; $t = 1.81$, $F = 2.75$	98.76 ± 1.04	1.3	98.7 [2]	98.65 ± 0.62
CIP tablets	99.4	99.39 ± 0.92; $t = 1.17$, $F = 2.14$	99.33 ± 0.78	1.9	97.2 [3]	97.21 ± 1.23

[a] The tabulated values of t and F at the 95% confidence limit are $t = 2.18$ and $F = 4.28$.

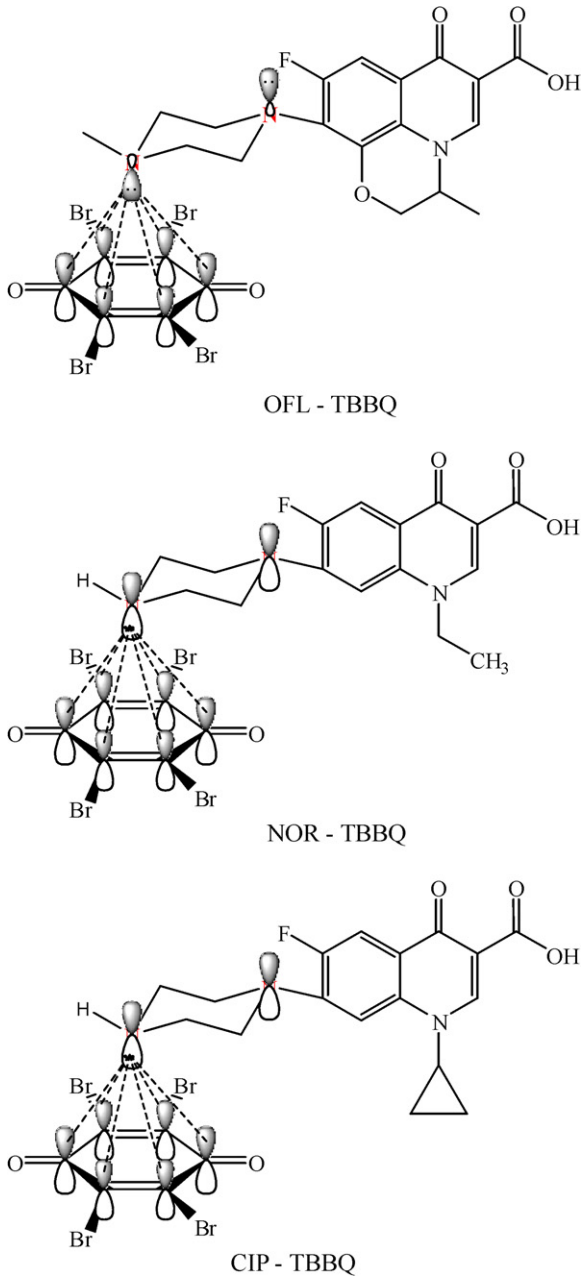


Fig. 9. The structural formula of CT complexes.

3.8. Analysis of pharmaceutical formulations

The proposed methods were applied to the examination of OFL and CIP in commercial tablets and NOR in capsules. Seven replicate examinations were made. Appropriate amounts of OFL, NOR, CIP sample solutions were transferred into three 10 ml cuvettes, and then subjected to the procedures described in Section 2.4.1. The sample contents were calculated according to the proposed method. A comparison was carried out between the proposed method and literature method, as well as the t-test and F-test. The results were as shown in Table 4. No significant difference for the t value and F value was observed between the two methods (confidence = 95%), which indicates similar precision and accuracy. The standard addition method was employed to test the validity of the method, which revealed a recovery in the range 98.76–99.91%.

4. Conclusions

Results acquired from this study clearly show that the spectrofluorimetric method was powerful and useful for the determination of three antibacterial quinolone derivatives, ofloxacin, norfloxacin and ciprofloxacin through charge-transfer complexation with 2,3,5,6-tetrabromo-1,4-benzoquinone. This proposed method was based on the reaction of these drugs as n-electron donors with the π-acceptor TBBQ and is more accurate and of high robustness with high recoveries. Another favourable characteristic of this method is that the absorbencies of the yellow products formed are stable at least for 24 h. Therefore, the method is simple, high sensitive, of good analytical selectivity and suitable for routine determination of the drugs.

Acknowledgements

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