Determination of Ofloxacin in Tablets by Room-Temperature Phosphorimetry on a Poly(vinyl alcohol) Solid Substrate

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An easy and sensitive method for the quantitative determination of ofloxacin (OFLX), a new fluoroquinolone antimicrobial agent, in a pharmaceutical formulation, tablet, was developed by using solid-substrate room-temperature phosphorimetry (RTP) on a poly(vinyl alcohol) substrate. The method did not require a dry gas flush during the measurement of phosphorescence. The influence of different conditions such as solution pH and concentrations of heavy atoms, used as the enhancer, were studied. The phosphorescence intensity of OFLX was enhanced using NaOH and KI as enhancers. A linear relationship between concentration and RTP intensity for each standard solution was obtained in the concentration range of 4—18000 ng/ml, and the determination limit was 4 ng/ml. The proposed method was applied to a determination of OFLX in a commercial tablet, and the results were compared with those of fluorescence and UV methods. It was proven that OFLX in a commercial tablet can be accurately measured by this method with a very small amount of sample solution.

Key words room-temperature phosphorimetry; poly(vinyl alcohol); solid substrate; ofloxacin

Solid substrate room-temperature phosphorimetry (SS-RTP) is widely known as a sensitive and simple analytical technique for determination of trace amounts of organic compounds. Among the utilizable substrate materials, filter paper is the most commonly used. Filter paper does, however, have some drawbacks; *e.g.*, it has considerable background emissions, the removal of which requires labor-intensive treatment, and continuous dry gas flow through the sample compartment of the spectrophotometer is necessary during measurement of RTP emissions because moisture and oxygen can quench emissions of the analytes. To overcome these drawbacks, we have investigated and report herein on a new solid substrate prepared from poly(vinyl alcohol) (PVA).[1] The best feature of this substrate is that it does not require any dry gas flush of the sample compartment during RTP measurement because the RTP intensity is very stable, even with atmospheric exposure. Furthermore, PVA has negligibly weak background emissions.[2]

The presence and/or amount of ofloxacin (OFLX), a new synthetic quinolone antibiotic with a broad spectrum of activity against gram-positive and gram-negative bacteria, is detected primarily by methods of fluorimetry and spectrophotometry, which are easily carried out.[3—7] Up to now, the most common technique for the determination of OFLX in pharmaceutical tablet has been based on HPLC with fluorescence or UV detection.[8—10] However, these methods need comparatively large amounts of sample solution. In order to develop a micro and trace analysis of OFLX by an easy method, we applied the PVA-substrate RTP to the analysis of OFLX.

Experimental

Materials and Reagents PVA, degree of polymerization 2000 and degree of hydrolysis 78—82 mol% (Kanto Chemical); OFLX (Sigma); sodium hydroxide, extra-pure grade (Kanto Chemical); thallium(I) nitrate, extra-pure grade (Kanto Chemical); and potassium iodide, extra-pure grade (Kanto Chemical), were used as purchased without further purification. Purified distilled water (Milli-Q, Millipore) was used as the solvent.

Solutions The solutions for the RTP measurement were prepared as follows. Stock solutions of OFLX were prepared in water or an aqueous NaOH solution of appropriate concentration. Stock solutions of NaOH, $TlNO_3$, and KI were prepared in water. Working solutions of lower concentration were obtained by an appropriate dilution of stock solution with water or the appropriate aqueous NaOH solution.

For the UV photometry, a stock solution of OFLX was prepared in water, and working solutions of lower concentration were obtained by appropriate dilution of stock solution with water.

For the fluorimetry, a stock solution of OFLX was prepared in a 49.55 mM phthalate buffer solution, and working solutions of lower concentration were obtained by an appropriate dilution of stock solution with the buffer solution.

For analysis of the commercial tablet, solutions were prepared as follows. Five tablets were accurately weighed and powdered. An amount of powder equivalent to one tablet was weighed and then appropriately diluted by aqueous NaOH solution, water, and 49.55 mM phthalate buffer solution for RTP, UV photometry, and fluorimetry, respectively. The solutions were used without filtration.

OFLX and KI solutions were stored in amber glass bottles covered with aluminum foil to shield them from the light.

Substrates The PVA granules were ground with a motor-driven mill, then sifted through a 250-mesh sieve. Next, 50 mg of the obtained fine powder, having a particle size of less than 63 μm, was pressed with a die normally used to make KBr disks for IR spectrophotometry. After the backside of the disk had been reinforced with cellophane tape, it was cut into 4×8-mm pieces that were stuck to a polyester plate (13×15 mm) with double-faced tape.

Procedure Prior to analyte spotting, 5 μl of a heavy-atom salt solution, used as an enhancer, was spotted alone the whole substrate surface. In cases in which this enhancer was not employed, the same volume of water was spotted. Then, 10 μl of analyte solution was spotted on the whole surface of the substrate. Spotting was performed with a microsyringe at 2-min intervals, providing the necessary standing times before the drying process to allow for sufficient penetration of the solutions into the substrate. The spotted substrate was laid on activated silica gel in a crucible that was placed in the center of a preheated microwave oven, then dried for 8 min. After drying, the substrate was stuck with double-faced tape to a laboratory-constructed sample holder. The RTP and signals were measured by a Hitachi F-4500 spectrofluorometer. Fluorescence signals were measured by a Hitachi F-4500 spectrofluorometer using a 1-cm cuvette. Excitation and emission slits for RTP measurement were set at 10 nm and these slits for fluorescence measurement were set at 5 nm. UV measurements were performed by a Shimadzu MultiSpec-1500 photodiode array spectrophotometer using a 1-cm cuvette.

Results and Discussion

Spectral Characteristics of OFLX RTP spectral characteristics of OFLX on the PVA substrate were investigated

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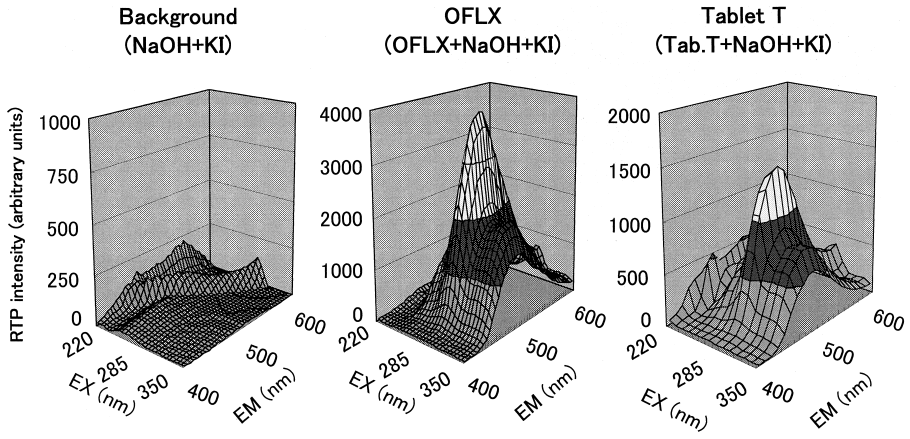

Fig. 1. Three-dimensional RTP Spectra of Background, OFLX, and Its Tablet
OFLX; 3.6 mg/l, Tablet T; 2.0 mg/l, NaOH; 0.1 M, KI; 0.8 M.

for both the OFLX standard and a commercial OFLX tablet. Figure 1 shows the three-dimensional phosphorescence spectra for each case.

In the background spectrum, broad and featureless bands of emission, probably arising from impurities in the NaOH, KI, and water, were observed, though these could be ignored due to their weakness. As for the OFLX measurement, RTP intensity of the background signal measured at the wavelength of peak maximum of OFLX ($\lambda_{ex,em}$=293, 497 nm) was around 9 and the analyte-to-background signal ratio at the point of determination limit was 2.

In the spectrum of the OFLX standard, one intense main emission peak was observed at $\lambda_{ex,em}$=293, 497 nm. Therefore, sensitive quantitative analysis for OFLX may be carried out by measuring the RTP intensity of OFLX on the PVA substrate.

The three-dimensional phosphorescence spectrum of the solution of the commercial tablet was measured without any pretreatment. As a result, a spectrum similar to that of the OFLX standard was observed, and no other contamination peaks were observed.

Figure 2 shows a comparison of excitation and emission spectra of OFLX with or without enhancers. As can be seen, the shape and maximum wavelength of the spectra did not change, even if NaOH and KI were added.

Stability of the RTP Intensity in the Atmosphere The RTP emission intensity changes of OFLX with or without enhancers with the passage of time after drying in the microwave oven are depicted in Fig. 3. In all cases, an initial increase followed by a flat plateau was observed. The initial growth of the RTP intensity was probably caused by the sample temperature being relatively high just after the sample was dried in the microwave oven, and then gradually falling to the ambient temperature. Namely, the phenomenon may be due to increase of analyte rigidity by shrink of the PVA substrate which originates from the falling down of the substrate temperature. However a more detailed investigation must be made in order to explain the nature of this phenomenon. In the cases of OFLX in the water and NaOH aqueous solution, the RTP intensities were very stable without affection of oxygen and moisture in the air. On the other hand, in the case of OFLX in the NaOH aqueous solution with KI, the RTP intensity slightly decreased with the passage of time. However,

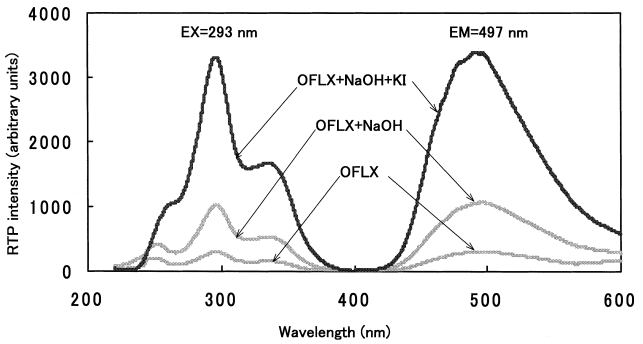

Fig. 2. RTP Excitation and Emission Spectra of OFLX with or without Enhancer(s)
OFLX; 1.0×10^{-5} M, NaOH; 0.1 M, KI; 0.8 M.

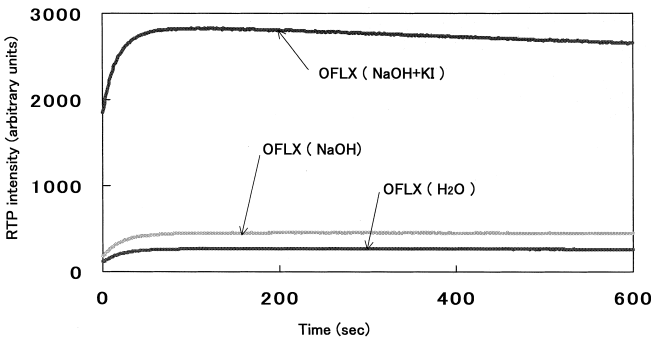

Fig. 3. Time-Dependent Stability of the RTP Intensity of OFLX in the Atmosphere
OFLX; 1.0×10^{-5} M, NaOH; 0.1 M, KI; 0.8 M. EX=293 nm, EM=497 nm.

it can be considered to be stable enough for a measurement in the air.

Effects of the pH Value of the OFLX Solution Changes in the RTP intensities of OFLX in acidic and basic mediums were studied, and the results are shown in Fig. 4. The acidic and basic OFLX solutions were prepared by using appropriate concentrations of aqueous HCl and NaOH solvents.

The pH variations caused no significant shift in the maximum OFLX RTP excitation and emission wavelengths. However, the intensities of the RTP signals were drastically affected by the solution pH values, especially in the basic

medium. In the acidic medium and at low NaOH concentrations in the basic medium, weak RTP intensities were obtained, and their reproducibility was inferior. In contrast, at high concentrations of NaOH in the basic medium, strong RTP intensities were obtained, with the degree of enhancement increaseing with NaOH concentration. The maximum RTP intensity was obtained with 1.0 M NaOH, but the reproducibility was inferior (R.S.D.=11.1%, n=5). Therefore, the most suitable NaOH concentration was determined to be 0.1 M NaOH, based on its superior reproducibility (R.S.D.=1.8%, n=5) and strong RTP intensity.

Heavy-Atom Effect The heavy-atom effect was investigated using two kinds of inorganic salts that are general RTP enhancers. We have previously reported that the RTP intensity is influenced by the deposition order of analyte and enhancer, and that a stronger RTP intensity is obtained using a spotting order of first the heavy-atom salt, and then the analyte.[11] As such, before deposition of the OFLX solution, several concentrations of aqueous KI and $TlNO_3$ solutions were deposited on the PVA substrate.

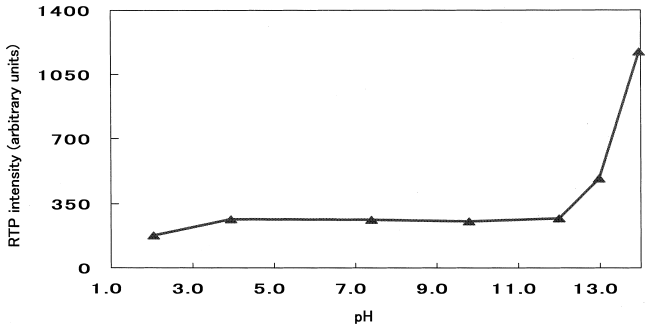

Fig. 4. Effects of Soluion pH Values on the RTP Intensity of OFLX

OFLX; 1.0×10^{-5} M, KI; 0.8 M. EX=293 nm, EM=497 nm. The pH values were adjusted using HCl and NaOH. Each value is the average of 5 determinations.

The RTP emission-intensity changes of OFLX in the presence of various amounts of KI and $TlNO_3$ with the passage of time are depicted in Fig. 5. It was confirmed that KI has a better enhancement effect than $TlNO_3$. The highest intensity was obtained for the 0.8 M KI, with this condition giving superior stability and reproducibility (R.S.D.=3.7%, n=5) of the RTP intensity than the other conditions employed. In this case, the RTP intensity was improved 5.5 times in comparison with that of the case without the KI. Solutions more concentrated than 0.8 M were found to reduce the RTP intensity, likely due to the excess KI causing a matrix prefilter effect and reducing the excitation light reaching the OFLX molecules. We therefore employed 0.8 M KI as an enhancer in this study.

Analytical Figures of Merit The relationship between the OFLX concentration and the RTP intensities was investigated under the optimized experimental conditions. The RTP intensities of OFLX reached a maximum on the time courses when the measurement was made at the wavelength of excitation and the emission maxima of OFLX. A calibration curve was constructed, with each of the RTP intensities being plotted as an average of 5 determinations. The calibration

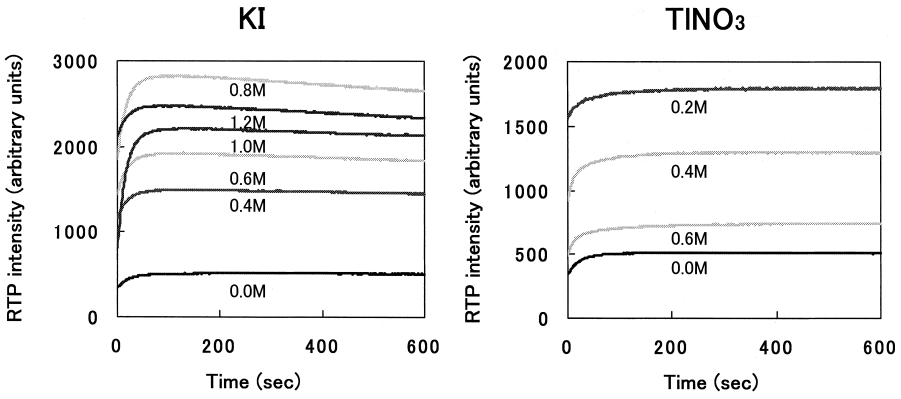

Fig. 5. Effects of KI or $TlNO_3$ amounts on the RTP Intensity of OFLX

OFLX; 1.0×10^{-5} M, NaOH; 0.1 M. EX=293 nm, EM=497 nm.

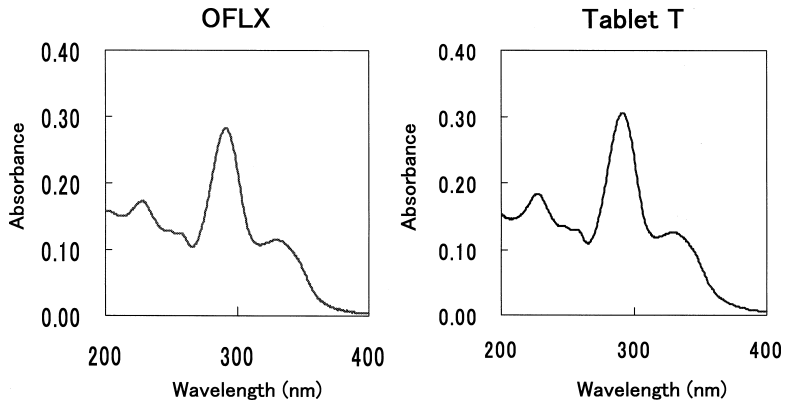

Fig. 6. UV Spectra of OFLX and Its Tablet

OFLX; 3.6 mg/l, Tablet T; 4.0 mg/l, Solvent; H_2O.

curve was linear from 4—18000 ng/ml, with a correlation coefficient of 0.994 and a determination limit, which was estimated from the lower limit of linear dynamic range, was 4 ng/ml. The R.S.D. of the five successive determinations was 5.60% at a level of 180 ng/ml. Therefore, highly sensitive quantitative analysis of OFLX could be performed using a very small volume, 5 μl, of sample solution.

UV Photometry and Fluorimetry UV photometry and fluorimetry have generally been used for analysis of OFLX. Compared to UV photometry, fluorimetry has the advantage of a relatively higher sensitivity. We therefore applied fluorimetry to the trace analysis of OFLX.

UV spectra of OFLX and the commercial OFLX tablet in water are shown in Fig. 6. In the spectrum of the OFLX standard, an intense main absorption peak can be seen at 292 nm. The absorption spectrum of the solution of commercial tablet was measured without any pretreatment. As a result, a spectrum similar to that of the OFLX standard was obtained, with no other contamination peaks being observed. These results suggest that the quantitative analysis of OFLX in a commercial tablet can be carried out.

A calibration curve was constructed, with each absorbance being plotted as an average of 3 determinations. The absorbance of OFLX was measured at 292 nm. A linear calibration curve was obtained over the working concentration range from 361—18100 ng/ml. The correlation coefficient for the linear fit was 0.9997. The R.S.D. of the five successive determinations was 0.37% at a level of 7230 ng/ml. These results indicate a good linearity between the absorbance and the OFLX concentrations.

Figure 7 shows the three-dimensional fluorescence spectra of the solvent of 49.55 mM phthalate buffer solution, OFLX standard, and commercial OFLX tablet. No significant emission bands can be observed in the background spectrum, thus confirming that the phthalate buffer does not interfere with the measurement. In the spectrum of OFLX standard, one intense emission peak can be observed at $\lambda_{ex,em}$=317, 503 nm. Therefore, sensitive quantitative analysis for OFLX may be able to be carried out by measuring the fluorescence intensity of OFLX. As described, the three-dimensional fluorescence spectrum of the solution of commercial tablet was measured without any pretreatment. Consequently, a spectrum similar to that of the OFLX standard is clearly observed with no other peaks from contamination. These results suggest that the quantitative analysis of OFLX in a commercial tablet can be carried out.

A calibration curve was constructed, with each of the fluorescence intensities being plotted as an average of 5 determinations. The fluorescence intensities of OFLX reached maximum values on the fluorescence spectra for which measurement was made at an excitation wavelength of 317 nm. A linear calibration curve was obtained with a correlation of 0.998 ranging from 10—10000 ng/ml. The R.S.D. of the five successive determinations was 1.02% at a level of 200 ng/ml. These results indicate a good linearity between the fluorescence intensity and the OFLX concentration. Although highly sensitive quantitative analysis of OFLX can be performed, the sensitivity of this method was slightly inferior to our proposed method. Another inferiority of the fluorescence method compared with our proposed method is it need for a much larger volume of sample solution.

Determination of OFLX in a Commercial Tablet For analysis of the commercial tablet, powdered tablets were diluted by aqueous NaOH solution, water, and 49.55 mM phthalate buffer solution for RTP, UV absorptiophotometry, and fluorimetry, respectively.

A comparison of the precision of the RTP, fluorimetric, and UV absorptiometric methods is given in Table 1. OFLX

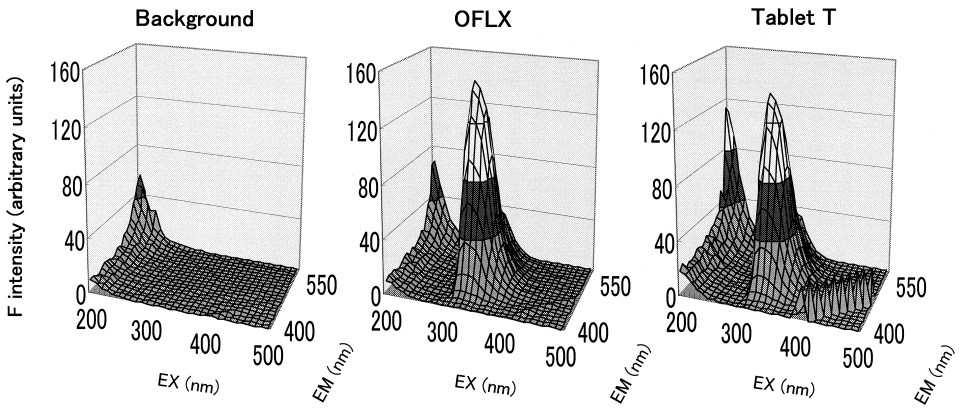

Fig. 7. Three-dimensional Fluorescence Spectra of Background, OFLX, and Its Tablet
OFLX; 4.0 mg/l, Tablet T; 4.0 mg/l, Solvent; Phthalate buffer (pH=4.01).

Table 1. Determination of OFLX in the Commercial Tablet by RTP, Fluorimetric and UV Absorptiometric Methods

	Method	Theoretical concentration (mg/l)	Mean found concentration (mg/l)	S.D.	R.S.D. (%)	Recovery (%)	*n*
Tablet T	RTP	0.40	0.40	0.034	8.61	99.50	5
	Fluorescence	0.40	0.42	0.007	1.55	105.00	5
	UV	4.00	3.99	0.032	0.81	99.63	5

Each tablet nominally contains 100 mg of OFLX.

was determined five times in commercial tablet forms. The R.S.D. values showed that fluorimetric and UV absorptiometric methods exhibited good reproducibility. However, R.S.D. value of the RTP method was slightly inferior to that of the other two methods, a point that must be improved in the future.

Recovery in the three methods was evaluated by analyzing a commercial OFLX tablet. The found value of OFLX measured by the RTP method was in good agreement with the theoretical value, thus proving that accurate determination of OFLX content in the tablet can be performed with a very small volume, 5 μl, of sample solution by the RTP method.

Conclusion

A new RTP method employing a PVA solid-substrate for sensitive analysis of a commercial tablet containing OFLX was developed. Strong RTP signals were obtained in NaOH solution in the presence of KI. These optimized experimental conditions allowed a very low limit of detection and a wide linear dynamic range. These results indicate that highly sensitive analysis can be conveniently carried out with a very small volume of sample solution.

The proposed method was applied to the analysis of OFLX in a commercial tablet, and it was proven that the measurement could be accurately performed with a very small volume of sample.

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