Spectrophotometric Determination of Quinolone Antibiotics by an Association Complex Formation with Aluminum(III) and Erythrosin

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A simple and sensitive spectrophotometric method for the determination of quinolone antibiotics was established based on an association complex formation with aluminum(III) and erythrosin. In the determination of ofloxacin as a quinolone antibiotic, Beer's law is obeyed in the range of 0.1 – 3.2 $\mu\text{g ml}^{-1}$, with an effective molar absorptivity at 555 nm and the relative standard deviation being $1.2 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ and 0.9% ($n = 6$). This method was successfully applied to the assay of quinolone antibiotics in pharmaceutical preparations.

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Ternary complexes can be classified into two major categories: a mixed-ligand complex type and an ion-association complex type. The modes of formation of both types of ternary complex, together with improvements in sensitivity and selectivity which accompany their formation, have been extensively discussed¹⁻⁵ in the field of inorganic analysis.

In one of the methods to increase the determination sensitivity of organic compounds, the binary ion-pair complex formation between an organic dye and an organic compound has often been used.⁶⁻⁸ The method is complicated since it requires an extraction procedure due to the formation of water-insoluble complexes. And the addition of a surfactant or water-miscible organic solvent to avoid to any extraction procedure usually causes decomposition of the ion-pair complex formed, and the organic compound cannot be determined any longer.

We have already reported⁹⁻¹¹ some simple and sensitive spectrophotometric methods for the determination of organic compounds by utilizing the ion-association complex formation among an organic compound, an organic dye, and a metal ion.

Quinolone antibiotics¹²⁻¹⁴ have recently attracted much interest, because they exhibit broad-spectrum bactericidal activity, excellent oral bioavailability, good tissue penetration and favorable safety and tolerability profiles. As all quinolone types¹⁵ with antibacterial activity have 4-quinolone nucleus with a nitrogen at position 1, a carboxyl group at position 3, and a ketone at position 4, absorption of orally administered quinolones is significantly decreased when these agents are coadministered with aluminum, magnesium, calcium, iron or zinc, because of the formation¹⁶⁻¹⁹ of insoluble drug cationic chelate compounds in the gastrointestinal tract. Methods for analyzing quinolones include spectrophotometry,²⁰⁻²² fluorometry,^{23,24} chromatography,²⁵⁻²⁹ electrophoresis,^{30,31} and electroanalysis.³²⁻³⁴

On the other hand, quinolone antibiotics complexation with aluminum(III), magnesium(II) and calcium(II) have been

studied by spectrophotometry.^{35,36} We noticed that an addition of acidic xanthene dyes such as erythrosin (ERY) in the reaction between a quinolone antibiotic and aluminum(III) showed a red shift as compared with that of the ERY-aluminum(III) solution, and that the magnitude of the increase in absorbance of the ERY-aluminum(III) solution was proportional to the concentration of a quinolone antibiotic. A method using ERY-aluminum(III) solution offers the advantages of greater sensitivity as compared with the conventional spectrophotometric methods.^{20,21}

In this work, a color reaction of quinolones by utilizing an ion-association complex formation among a quinolone, a metal ion and an organic dye was studied in aqueous media, and then suitable conditions for the spectrophotometric determination of quinolones by using aluminum(III) and ERY were established. The proposed method was applied to the assays of these drugs in pharmaceutical preparations.

Experimental

Reagents, solutions and apparatus

Reagents and solutions. Ofloxacin (OFX) and other quinolone antibiotics (norfloxacin, enoxacin and levofloxacin), analytical-grade reagents, were purchased from Sigma-Aldrich Co., and used without further purification. The stock solutions ($1.0 \times 10^{-2} \text{ mol L}^{-1}$) of quinolones were prepared by dissolving appropriate quantities of each quinolone in small amounts of 1 mol L⁻¹ sodium hydroxide solution and were diluting to 100 ml with water. The working solutions were made by diluting these stock solutions as required. These solutions were stored in a cool (4°C) and dark place. An aluminum(III) solution was prepared by dissolving aluminum plate (99.99%, Wako Pure Chemical Co., Ltd.) in 0.1 mol L⁻¹ hydrochloric acid. A solution of erythrosin (ERY) was prepared by dissolving of ERY (Wako Pure Chem. Co., Ltd.) in water. A 0.25% methylcellulose (MC) solution was prepared by dissolving MC (350 – 550 cps, Kishida Chemical Co., Ltd.) in cold water. A buffer solution of pH 5.4 was made by mixing appropriate amounts of a 0.2 mol dm⁻³ acetic acid and 0.2 mol L⁻¹ sodium acetate solutions. All other materials and reagents were of analytical reagent grade and were

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Table 1 Effect of dyes

Dye	Absorbance	$\lambda_{\text{max}}/\text{nm}$
ERY	0.619	555
Eosin Y	0.121	535
Eosin B	0.045	545
Methyleosin	0.221	548
Ethyleosin	0.095	548
Phloxine B	0.092	568
2,4,5,7-Cl FI ^a	0.112	540

OFX, 5.0×10^{-6} M; Al(III), 4.0×10^{-4} M; dye solution, 1.0×10^{-4} M; MC, 0.025%; pH, 5.4; reference, Al(III)-dye solution.

a. 2,4,5,7-Tetrachlorofluorescein.

used without further purification. Pure water was prepared by purifying deionized water with a Milli-Q Labo system just before use.

Apparatus. A Shimadzu spectrophotometer (Model UV-160) with 1.0-cm matched silica cells was used for an absorbance measurements. The pH measurements were made with a Horiba (F-51) pH meter in combination with a calomel glass electrode.

Standard procedure for the determination of quinolones

An aliquot of each quinolone solution was transferred to a 10-ml volumetric flask. To this solution, 1.0 ml of a 4.0×10^{-3} mol L⁻¹ aluminum(III) solution, 2.0 ml of the buffer solution, and 1.0 ml of a 1.0×10^{-3} mol L⁻¹ ERY solution were added. The mixture was diluted to 10 ml with water, transferred into a test tube, mixed well and kept at room temperature for 20 min. The absorbance of the resultant solution (Solution A) was measured at 555 nm against a reagent blank solution (Solution B) prepared in the same way.

Results and Discussion

Choice of metal ion and dye

In order to examine the utility of the method for the determination of quinolones based on ternary complex formation among a quinolone, a dye and a metal, we studied the color reactions between various metal ions and xanthene dyes by comparing the situations in the presence of OFX as a quinolone. First, the effect of metal ions on the reaction system was investigated. Only aluminum(III) was effective among various metal ions; aluminum(III), iron(III), zinc(II), copper(II), zinc(II), nickel(II), magnesium(II) and calcium(II). The reason why only aluminum(III) is effective is probably that the stability constant³⁷ of the OFX-aluminum(III) complex is large. Next, the xanthene dyes were studied by using ERY, eosin Y, eosin B, methyleosin, ethyleosin, phloxine B and 2,4,5,7-tetrachlorofluorescein. ERY was best in sensitivity, as is shown in Table 1.

Optimization of reaction variables

The effect of the pH on the reaction was examined by using various buffer solutions, such as 0.2 mol L⁻¹ acetic acid/0.2 mol L⁻¹ sodium acetate, 0.1 mol L⁻¹ citric acid/0.1 mol L⁻¹ sodium citrate, 0.1 mol L⁻¹ succinic acid/0.05 mol L⁻¹ borax, 0.1 mol L⁻¹ citric acid/0.05 mol L⁻¹ disodium hydrogenphosphate, and 0.1 mol L⁻¹ acetic acid/0.05 mol L⁻¹ EDTA·2Na solutions. Maximum and constant absorbance was achieved over the pH range 4.8 – 5.7 by using 2.0 ml of 0.2 mol L⁻¹ acetic acid/0.2 mol L⁻¹ sodium acetate buffer solution.

Solution A without surfactant was unstable and gave a

Table 2 Effect of surfactants

Surfactant	Absorbance	λ_{max}
MC (350 – 550 cps)	0.619	555
MC (15 cps)	0.596	553
MC (25 cps)	0.586	555
MC (1000 – 1800 cps)	0.603	553
Tween 20	0.051	545
Tween 80	0.053	543
Brij 78	0.047	545
PVP (K-30)	0.049	541
PVP (K-90)	0.051	545
PVA	0.408	557
Triton X-100	0.064	544
STAC	0.051	540
SDS	0.277	550
Swanol AM-301	0.439	573

OFX, 5.0×10^{-6} M; Al(III), 4.0×10^{-4} M; ERY, 1.0×10^{-4} M; surfactant, 0.025%; pH, 5.4; reference, Solution B.

precipitate. Thus, to stabilize the color development and to enhance the sensitivity, we examined the effects of different surfactants: nonionic (MC, polyoxyethylene(20)sorbitan monolaurate (Tween 20), polyoxyethylene(20)sorbitan monooleate (Tween 80), polyoxyethylene(20)stearyl ether (Brij 78), *N*-polyvinylpyrrolidone (PVP), polyvinylalcohol (PVA), polyoxyethylene octyl phenyl ether (Triton X-100)), cationic (stearyltrimethylammonium chloride (STAC)), anionic (sodium dodecylsulfate (SDS)), and amphoteric (lauryldimethylaminoacetic acid betaine (Swanol AM-301)). As is shown in Table 2, MC (350 – 550 cps) was best as a dispersion agent; the maximum and constant absorbance of Solution A was observed upon the addition of more than 0.4 ml of a 0.25% MC solution to the final volume of 10 ml.

The effects of the concentrations of ERY and aluminum(III) were examined. A maximum and constant absorbance was observed upon the addition of more than 4.0 ml of 1.0×10^{-3} mol L⁻¹ aluminum(III) solution, while maintaining a fixed final concentration of ERY (1.0×10^{-4} mol L⁻¹). Further experiments were carried out with 1.0 ml of 4.0×10^{-3} mol L⁻¹ aluminum(III) solution and 1.0 ml of 1.0×10^{-3} mol L⁻¹ ERY solution in the final volume of 10 ml in consideration of the absorbance of Solution B.

The same effect of absorbance value was obtained even when the order of the addition of the reagents was varied. The absorbance of Solution A against Solution B was immediately stabilized, and the absorbance value was almost constant for at least 24 h at room temperature. Therefore, 20 min of standing time at room temperature was adopted for all measurements.

Figure 1 shows the absorption spectra of Solutions A and B under the standard procedure.

Calibration curves

A calibration curve for OFX was constructed by the standard procedure. A good linear relationship was observed over 0.1 – 3.2 $\mu\text{g ml}^{-1}$ of OFX. The effective molar absorptivity (ϵ) was calculated from the slope of the calibration graph to be 1.2×10^5 L mol⁻¹ cm⁻¹. This procedure is much more sensitive than the methods using bromophenol blue²¹ ($\epsilon = 1.1 \times 10^4$), bromothymol blue²¹ ($\epsilon = 2.0 \times 10^4$) and supracene violet 3B²⁰ ($\epsilon = 1.1 \times 10^4$). The relative standard deviation (RSD) for six runs of 1.8 $\mu\text{g ml}^{-1}$ of OFX was 0.9%. The calibration curves were also constructed for the optimum conditions for other quinolone and tetracycline antibiotics: nolfloxacin, enoxacin, levofloxacin, tetracycline, minocyclone and doxycycline. The analytical data, e.g., the effective molar absorptivity (ϵ), the linear range ($\mu\text{g ml}^{-1}$),

Fig. 1 Absorption spectra. OFX, 5.0×10^{-6} M; Al(III), 4.0×10^{-4} M; ERY, 1.0×10^{-4} M; MC, 0.025%; pH, 5.4. Curve A, OFX-Al(III)-ERY solution (Solution A); curve B, Al(III)-ERY solution (Solution B), OFX-ERY solution and ERY solution; curve C, curve A minus curve B.

Table 3 Reactivities of other drugs with this method

Drug	Molar absorptivity/ $\times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$	Linear range/ $\mu\text{g ml}^{-1}$	<i>r</i>	RSD, ^a %
OFX	1.2	0.1 – 3.2	0.996	0.9
Norfloxacin	1.7	0.05 – 2.0	0.998	0.7
Enoxacin	1.1	0.1 – 2.6	0.997	0.8
Levofloxacin	1.1	0.1 – 3.2	0.995	0.9
Tetracycline	1.0	0.1 – 4.1	0.999	0.8
Minocycline	1.4	0.05 – 3.7	0.999	0.7
Doxycycline	1.0	0.1 – 4.1	0.997	0.8

Al(III), 4.0×10^{-4} M; ERY, 1.0×10^{-4} M; MC, 0.025%; pH, 5.4; reference, Solution B.

a. Drug, 5.0×10^{-6} M (*n* = 6).

the correlation coefficient (*r*), and RSD for these antibiotics are presented in Table 3.

Interference of foreign substances

Various amounts of foreign substances were added to a fixed amount of OFX ($1.8 \mu\text{g ml}^{-1}$). There was no interference under the following conditions: 50- to 100-fold molar ratios of sodium, potassium, ammonium, calcium(II), magnesium(II), zinc(II), copper(II), manganese(II), chloride, nitrate, sulfate, glucose, caffeine, glycine, ascorbic acid and urea; 2- to 5-fold molar ratios of phosphate, citric acid, oxalic acid, thiamine and pyridoxine; thiamine and pyridoxine as organic bases reacted with ERY to form colored complexes. Iron(III) and fluoride ions interfered in equimolar ratio of OFX; as fluoride ion belongs to the hard bases, a stable complex might form with aluminum(III) as a hard acid. The results are summarized in Table 4.

Application

The proposed method was applied to the determination of OFX and other quinolones in tablets. The contents of the tablets were accurately weighed and ground in a mortar to a fine powder. The requisite volume of powder was weighed, transferred into a 100-ml volumetric flask, diluted to the mark with water and filtered. An appropriate amount of the sample solution was taken and determined according to the standard procedure. Satisfactory results were obtained for three drugs. Moreover, to check the validity of the proposed method, we

Table 4 Effect of foreign substances

Substance	$\mu\text{g/ml}$	Molar ratio (substance/OFX)	Absorbance at 555 nm	Recovery, %
None	—	—	0.619	100.0
Ca(II)	20.0	100	0.619	100.0
Zn(II)	16.4	50	0.619	100.0
Mg(II)	6.1	50	0.619	100.0
Mn(II)	5.5	20	0.619	100.0
Fe(II)	0.3	1	0.619	100.0
Fe(III)	0.3	1	0.699	113.0
KNO ₃	50.6	100	0.619	100.0
NH ₄ Cl	26.7	100	0.619	100.0
Na ₂ SO ₄	71.0	100	0.619	100.0
Na ₂ HPO ₄	3.5	5	0.619	100.0
NaF	0.2	1	0.583	94.2
Ascorbic acid	88.1	100	0.619	100.0
Maleic acid	58.0	100	0.619	100.0
Succinic acid	29.5	50	0.619	100.0
Citric acid	5.3	5	0.619	100.0
Oxalic acid	3.2	5	0.619	100.0
Glucose	90.0	100	0.619	100.0
Lactose	180.2	100	0.619	100.0
Sucrose	171.2	100	0.619	100.0
Urea	30.0	100	0.619	100.0
Glycine	37.5	100	0.619	100.0
Caffeine	53.1	50	0.619	100.0
Thiamine	8.2	5	0.718	116.0
Pyridoxine	5.1	5	1.083	175.0
H ₂ O ₂	3.4	20	0.619	100.0
HSA	4.0	—	0.619	100.0

OFX, 5.0×10^{-6} M; Al(III), 4.0×10^{-4} M; ERY, 1.0×10^{-4} M; MC, 0.025%; pH, 5.4; reference, Solution B.

Table 5 Assay of pharmaceutical preparations and recoveries

Sample	Content/mg ^a		RSD, ^{b,c} %	Recovery, ^{b,c} %
	Nominal amount	Present method		
A	100	100.3	1.2	98.1
B	100	104.6	1.8	98.0
C	200	100.8	1.5	99.5

A, OFX tablet; B, levofloxacin tablet, C, norfloxacin tablet.

a. mg per 1 tablet.

b. Mean of 3 determinations.

c. $0.9 \mu\text{g ml}^{-1}$ of OFX, $1.0 \mu\text{g ml}^{-1}$ of levofloxacin and $0.9 \mu\text{g ml}^{-1}$ of norfloxacin were taken in final concentration of each drug.

applied the standard addition method by adding OFX, levofloxacin and norfloxacin to the previously analyzed tablets. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of pure drugs, and the recoveries of added quantity were found to be more than 98%. This indicates that the proposed method gives accurate results. These results are given in Table 5.

Study of composition of the colored complex

In order to clarify the reaction mechanism, we measured the absorption spectra of ERY, Al(III)-ERY, OFX-ERY, OFX, OFX-Al(III) solutions. As a result, the three absorption spectra of ERY, Al(III)-ERY and OFX-ERY solutions completely agreed with each other, but the absorption spectrum of OFX-Al(III) solution differed from that of OFX solution, as shown in Fig. 2.

Fig. 2 Absorption spectra between {OFX-Al(III)} solution and OFX solution. OFX, 1.0×10^{-4} M; Al(III), 1.0×10^{-4} M; MC, 0.025%; pH, 5.4. Curve A, OFX-Al(III) solution; curve B, OFX solution.

Furthermore, we studied the composition of the colored complex by Job's method of continuous variation and by the molar ratio methods. The Al(III)-to-OFX ratio and the {Al(III)/OFX}-to-ERY ratio were 1:1 and 1:1, respectively. On the other hand, ERY exists in one of the following forms, depending on the pH of the aqueous solution:

where R denotes the undissociated parts of ERY. It was found³⁵ that $\text{p}K_{a2}$ and $\text{p}K_{a3}$ were 4.26 and 7.48, respectively, and the chemical form of ERY at pH 5.4 is estimated to be HR^- . In addition, as the amount of methanol or ethanol was increased, the color development of Solution A decreased. From these results, we deduced that the colored ternary complex formed in this reaction system was an ion-association complex between {Al^{III}(OFX)} cation and (ERY) anion.

In conclusion, a simple and sensitive spectrophotometric method for quinolone antibiotics such as OFX was established based on an association complex formation with aluminum(III) and ERY. This method was applicable to the measurement of OFX and other quinolone antibiotics in pharmaceutical preparations (tablet). The sensitivity ($\epsilon = 1.2 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$) of OFX by the proposed method was more sensitive than those of other spectrophotometric methods.^{20,21} In addition, there was a remarkable improvement in the influences of foreign substances. The present method, owing to no need for solvent extraction, was applied to assays of quinolone antibiotics in pharmaceutical preparations with satisfactory results. Though further investigations are necessary to confirm the application of this method to a variety of samples, including application to HPLC, the developed procedure is suitable for the routine analysis of quinolone antibiotics in real samples.

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