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Spectrophotometric determination of pefloxacin in pharmaceutical preparations*

M. JELIKIĆ-STANKOV,†‖ D. VESELINOVIĆ,‡ D. MALEŠEV§ and Z. RADOVIĆ§

†*Institute of Analytical Chemistry, Faculty of Pharmacy, Belgrade, Yugoslavia*
‡*Institute of Physical Chemistry, Faculty of Science, Belgrade, Yugoslavia*
§*Institute of Physical Chemistry, Faculty of Pharmacy, Belgrade, Yugoslavia*

Abstract: It has been established that the antibiotic pefloxacin (Abaktal) methanesulphonate reacts with Fe(III) at pH 1.00–8.00 to form a water-soluble complex with maximum absorbance at 360 nm. The composition of the complex, determined spectrophotometrically by the application of Job's, molar-ratio and Bent–French's methods, was pefloxacin: Fe(III) = 1:1 (pH = 2.50; λ = 360 nm; μ = 0.1 M). The relative stability constant, obtained by the methods of Sommer and Asmus was $10^{5.02}$ (pH = 2.50; λ = 360 nm; μ = 0.1 M). The molar absorptivity of the complex at 360 nm was found to be 4.8×10^3 l mol^{-1} cm^{-1}, Beer's law was followed for pefloxacin concentrations of 2.15–85.88 μg ml^{-1}. The lower sensitivity limit of the method was 2.15 μg ml^{-1}. The relative standard deviation (n = 10) was 0.57–1.07%. The method can be applied to the rapid and simple determination of pefloxacin in aqueous solutions and tablets.

Keywords: *Antibiotic; Abaktal; pefloxacin; iron(III) nitrate; complex; spectrophotometry.*

Introduction

Pefloxacin (Abaktal) [1-ethyl-6-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid] in the form of its methanesulphonate (mesylate dihydrate) is a new synthetic antibiotic from the group of 4-quinolones, which [1] makes possible the successful treatment of serious infections caused by resistant groups of bacteria. The drug has a wide antimicrobial spectrum and its oral use has simplified the treatment of infections which had been previously dealt with only parenterally [2–5].

In addition to the use of microbiological methods, pefloxacin can be successfully determined by a chemical method based on measurements of the absorbance of the complex formed between the drug and Fe(III). The present work is part of a systematic investigation on complexation reactions of antibiotics with metal ions and on their applications to the assay of antibiotics in pharmaceutical formulations.

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‖To whom correspondence should be addressed.

Experimental

Reagents

Pefloxacin methanesulphonate dihydrate (purity 99.64%) was synthesized in the laboratories of the pharmaceutical company Lek (Ljubljana, Yugoslavia). Abaktal tablets (Lek, Ljubljana) each contained 400 mg of pefloxacin. Other reagents were analytical grade (Merck). Double-distilled water was used for the preparation of solutions.

Solutions

(10^{-3} M) Pefloxacin was used as a stock solution which was found to be stable for several days. A second standard solution made from Abaktal tablets was prepared by dissolving a portion of powdered tablets (10) in double-distilled water; the solution was filtered into a standard volumetric flask and diluted with water to give a final nominal concentration of 10^{-3} M.

The stock Fe(III) nitrate solution was standardized gravimetrically. 2×10^{-3} M Fe(III) nitrate used for the determination of pefloxacin was prepared by dissolving Fe(III) nitrate in 0.0158 M nitric acid. The ionic strength (μ) of the solution was kept constant (μ = 0.1 M) with 1 M $NaNO_3$.

Apparatus

A Pye Unicam SP 6-500 ultraviolet-visible spectrophotometer (Cambridge, UK) equipped with 1-cm cuvettes, was used. A Radiometer pH 62 pH meter was used for pH measurements with a combined electrode system. For the determination of pefloxacin an Abott-spectrum analyzer (USA) was employed.

Procedure for calibration curve

To a measured volume of pefloxacin standard solution (0.1–1.5 ml) or Abaktal tablets solution (0.10–0.30 ml) was added 2.00 ml of 4×10^{-3} M Fe(III) nitrate in nitric acid and 1.0 ml of 1 M $NaNO_3$. The solution was diluted to 10 ml (pH 2.50) and shaken well; the absorbance was measured at 404 nm using 4×10^{-3} M Fe(III) nitrate as the reference solution. All measurements were made at room temperature (25 ± 0.5°C).

Results and Discussion

Absorption spectra

The complexation reaction of pefloxacin with Fe(III) was followed at pH 2.00–8.00, and the absorption spectra were recorded at 340–420 nm. It has been found that pefloxacin and Fe(III) form a complex with a maximum absorbance at 360 nm (Fig. 1, curve 3). Under the same experimental conditions the drug absorbs within the range of the absorption of the complex whereas the absorbance of Fe(III) nitrate solution is negligible. All experiments were carried out with reference to pefloxacin solution.

Effect of pH on complex formation

By following the effect of pH on complex formation it has been established that at pH values less than 1.00, the complex is not formed. At pH 2.50, a maximum absorbance due to the complex appears at 360 nm (Fig. 2). On further increase of pH, the wavelength of absorbance does not shift but the absorbance of the complex increases up

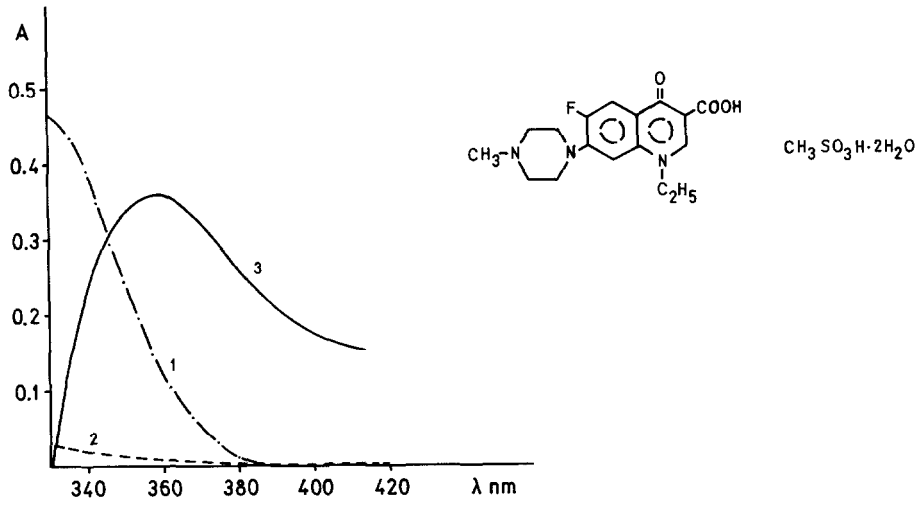

Figure 1
Absorption spectra of pefloxacin (curve 1); Fe(III) nitrate (curve 2); and the complex (curve 3). [Pefloxacin] = 8×10^{-5} M; [Fe(III)] = 4×10^{-5} M; pH 3.20; $\mu = 0.1$ M.

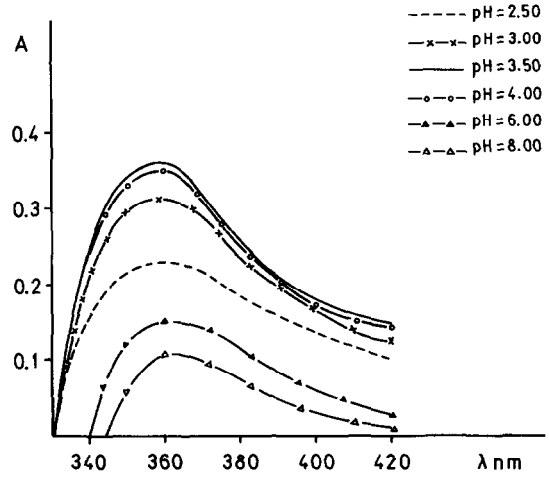

Figure 2
The effect of pH on complex formation. [Pefloxacin] = 8×10^{-5} M; [Fe(III)] = 4×10^{-5} M; $\mu = 0.1$ M.

to a maximum at pH 3.5. At higher pH values the complex absorbance decreases probably because of the concurrent reaction of Fe(III) hydrolysis. In the determination of pefloxacin Fe(III) nitrate is used in excess; in order to diminish the effect of Fe(III) hydrolysis on the complexation reaction, the determination was carried out at a pH one unit lower than that at which the complex exhibits its maximum absorbance.

Optimum conditions of complex formation

The effect of Fe(III) concentration shows that a five-fold excess of the reagent leads to a quantitative conversion of pefloxacin into the complex. The use of a higher reagent

conversion does not produce an increase in absorbance. The complex is formed immediately; its absorbance remains unchanged for 20 h, but then begins to decrease. The effect of ionic strength on complex formation was followed in the range 0.1–1.0 M. At an ionic strength of 0.1 M optimally shaped spectra were obtained.

Composition of the complex

The stoichiometric ratio of pefloxacin to Fe(III) in the complex was determined by the application of the Job's method of continuous variations [6, 7]. The curve obtained (Fig.

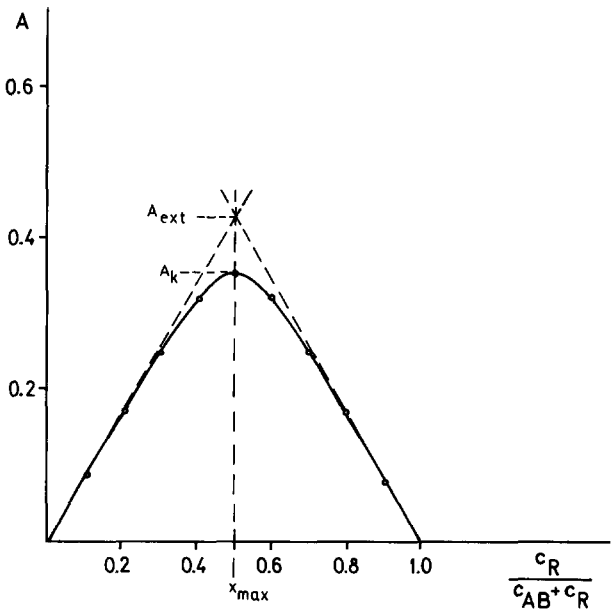

Figure 3
Job's curve of equimolar solutions at 360 nm. [Pefloxacin] + [Fe(III)] = 2×10^{-4} M; pH 2.50; μ = 0.1 M; pefloxacin (Abaktal) = AB.

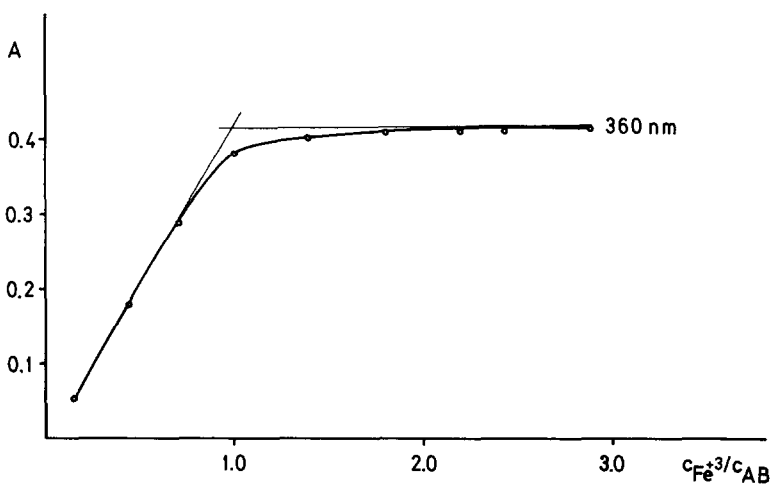

Figure 4
Molar ratio method. [Pefloxacin] = 7×10^{-5} M; pH 2.50; μ = 0.1 M; pefloxacin (Abaktal) = AB.

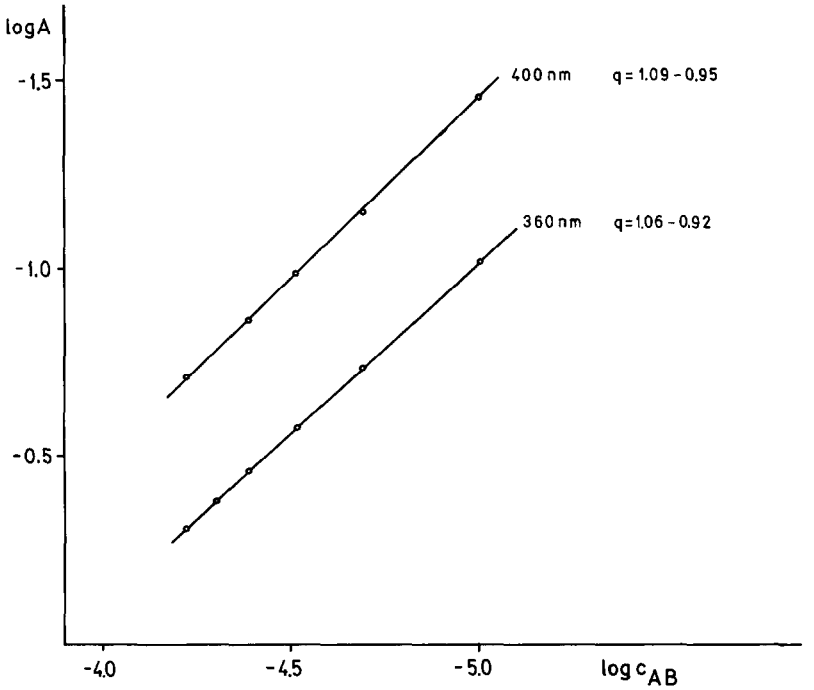

Figure 5
Bent–French's method. [Pefloxacin] = 1×10^{-5}–6×10^{-5} M; [Fe(III)] = 2×10^{-4} M; pH 2.50; μ = 0.1 M; pefloxacin (Abaktal) = AB.

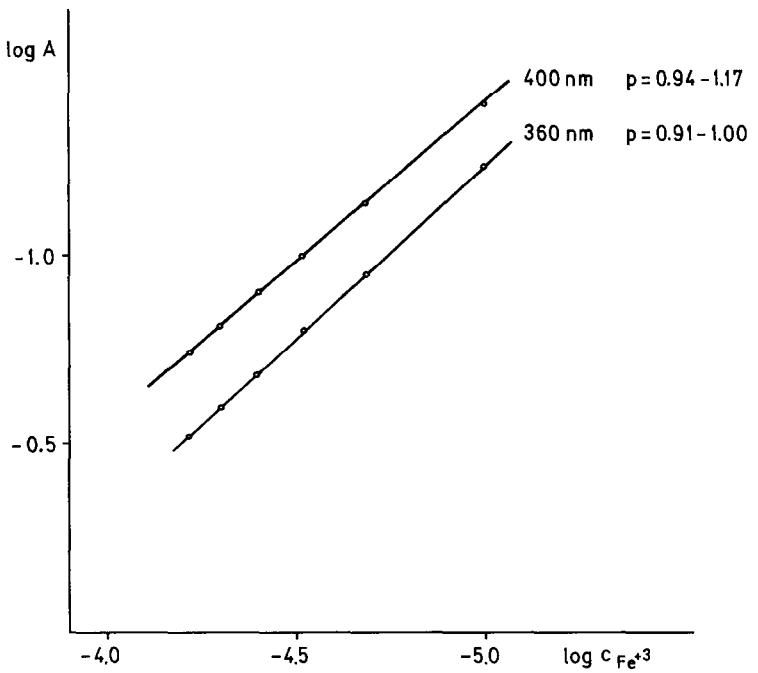

Figure 6
Bent–French's method. [Pefloxacin] = 8×10^{-5} M; [Fe(III)] = 1×10^{-5}–6×10^{-5} M; pH 2.50; μ = 0.1 M.

3) had a maximum at a molar ratio of $x_{max} = 0.5$ which indicated the formation of a 1:1 complex. By the molar ratio method [8] a curve was obtained with an intercept at the molar ratio pefloxacin:Fe(III) = 1:1 (Fig. 4). The composition of the complex was confirmed also by the method of Bent–French [9] (Figs 5 and 6). The determination of the composition and relative stability constant of the complex was carried out under the same experimental conditions as those used in the spectrophotometric method (pH = 2.5; μ = 0.1 M).

Relative stability constant of the complex

The relative stability constant (K') of the complex was determined by the methods of Sommer [10] and Asmus [11]. The results obtained are shown in Tables 1 and 2. The K' values obtained by the two methods are in good agreement.

Quantification of Beer's law

A linear relationship between absorbance and concentration of pefloxacin was established over the range 1×10^{-5}–1.5×10^{-4} M. The molar absorptivity of the complex was found to be 4.8×10^3 l mol^{-1} cm^{-1}. The regression equation was $y =$

Table 1
Relative stability constant of the pefloxacin complex calculated according to Sommer's method*

log K'	log K'_{min}	log K'_{max}	SD†	RSD‡ (%)
5.02	4.98	5.04	0.04	0.75

*Conditions: pH 2.50; μ = 0.1 M; $T = 25 \pm 0.5$°C; $n = 10$.
†SD = Standard deviation.
‡RSD = Relative standard deviation.

Table 2
Relative stability constant of the pefloxacin complex calculated according to Asmus' method*

log K'	A_k	A_{ext}
5.07	0.350	0.430

*Conditions: pH 2.50; μ = 0.1 M; $T = 25 \pm 0.5$°C.

Table 3
Spectrophotometric determination of pefloxacin mesylate in Abaktal tablets with Fe(III) nitrate*

Pefloxacin mesylate	0.00465 mg ml^{-1}	0.00926 mg ml^{-1}	0.01369 mg ml^{-1}
Found $\bar{x}$	0.00468	0.00926	0.01397
s_{min}	0.00462	0.00916	0.01381
x_{max}	0.00475	0.00934	0.01410
SD†	0.00005	0.00007	0.00008
RSD‡ (%)	1.07	0.76	0.57

*Conditions: λ = 404 nm; pH 2.50.
†SD = Inter-assay standard deviation ($n = 10$).
‡RSD = Relative standard deviation.

$5.859x + 1.326 \times 10^{-3}$. The correlation coefficient (r) was 0.9998, indicating good linearity. The relative standard deviation was 0.6–0.80% for pefloxacin concentrations of 0.00465–0.01396 mg ml^{-1} (Table 3).

Analysis of pharmaceutical preparations

The proposed method was applied to the determination of pefloxacin in Abaktal tablets. The presence of excipients did not affect the determination. Because the method developed is rapid and simple and the results are reproducible, it can be recommended for the routine analysis of pefloxacin in aqueous solutions and tablets.

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