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The effect of pH, buffer type and drug concentration on the photodegradation of ciprofloxacin

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Abstract

The photochemical behaviour of ciprofloxacin was investigated in the pH range 3.0–10.6 in solutions irradiated with a high-pressure mercury lamp at 313 nm. The reaction was followed by an isocratic reversed-phase high-performance liquid chromatographic method. Ciprofloxacin was most sensitive to photodegradation at slightly basic pH, where the drug is in zwitterionic form. The stability increased considerably when the pH was lowered towards 3–4. The reaction rate was inversely proportional to the initial drug concentration, but it was not affected by buffer type (acetate, citrate and phosphate at pH 5.0). Two major degradation products were formed in acidic solutions. In solutions at pH $\geq$ 6.0, several additional degradation products were detected, which underwent secondary degradation on longer exposure to radiation.

Keywords: Ciprofloxacin hydrochloride; Photodegradation; HPLC; pH; Concentration; Buffer species

1. Introduction

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinolone-3-carboxylic acid), shown in Fig. 1, is a synthetic antibiotic chemically related to nalidixic acid, the first generation DNA gyrase inhibitor. In ciprofloxacin, the interaction of the C-6 fluorine and the C-7 piperazine exhibits enhanced Gram-negative and Gram-positive antibacterial activity, including activity against *Pseudomonas aeruginosa* and staphylococci. This fluoroquinolone has been in worldwide use since the mid-1980s (Harold, 1987; LeBel, 1988; Stein, 1988).

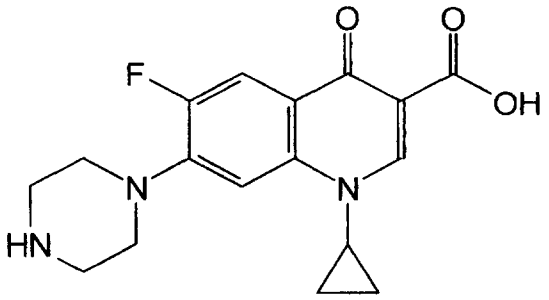

Fig. 1. Structure of ciprofloxacin.

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Soon after its introduction in common clinical practice, nalidixic acid became linked with phototoxic reactions (Zelickson, 1964; Birkett et al., 1969) and photosensitivity (Baes, 1968). Ciprofloxacin became similarly linked 20 years later, though with lower incidence of cases (Ball, 1986; Ferguson and Johnson, 1990). The loss of antibiotic activity in irradiated ciprofloxacin solutions has been reported in in vivo studies (Ferguson et al., 1988). A wavelength-dependent loss of antibiotic activity in ciprofloxacin solutions stressed with ultraviolet and visible radiation in vitro has been observed, with maximal effect around 320 nm (Phillips et al., 1990). Official guidelines (European Pharmacopeia, 1994; US Pharmacopeia/National Formulary 23/NF 18, 1995) recommend light-protection for ciprofloxacin and its liquid formulations during storage. Although a few publications have dealt with ciprofloxacin photodegradation (Tammilehto et al., 1994; Tiefenbacher et al., 1994; Torniainen and Mäki, 1995), there are no detailed studies on reaction kinetics and the products formed under different conditions.

The study presented here is a sequel to our development of analytical methods for monitoring the photodegradation of ciprofloxacin in aqueous solutions (Tammilehto et al., 1994; Torniainen and Mäki, 1995). The effect of pH, buffer type and concentration of ciprofloxacin on the reaction kinetics and product profiles in irradiated solutions was monitored by reversed-phase high-performance liquid chromatography (HPLC). Comparison was made with solutions stressed under daylight.

2. Materials and methods

2.1. Materials

The identity and purity of ciprofloxacin hydrochloride Ph.Eur. were verified by HPLC, by thin-layer chromatography (TLC), by measuring the melting point (Electrothermal digital melting point apparatus, Southend, UK) and by ultraviolet (UV) and infrared (IR) spectrometry (Philips PU 8740 UV/VIS spectrometer and Unicam SP3-200 infrared spectrometer, both from Pye Unicam Ltd., Cambridge, UK).

All chemicals and solvents were of analytical or HPLC grade. The components of actinometric and buffer solutions were obtained from E. Merck (Darmstadt, Germany), except acetic acid 100% which was from Prolabo Groupe Rhone-Poulenc (Manchester, UK). For HPLC, the acetonitrile was purchased from Rathburn Chemicals (Walkerburn, UK); 1-heptanesulphonic acid sodium salt (Na-HSA) from Sigma (St. Louis, MO, USA) and *o*-phosphoric acid from E. Merck. HPLC water was processed with an Alpha-Q water purification system from Millipore (Molsheim, France) and distilled water with a Finn-Aqua H75 Santasalo-Sohlberg device (Espoo, Finland).

2.2. HPLC systems and methods

The following apparatus from Waters Associates (Milford, MA, USA) was used for quantitation of ciprofloxacin: a model 501 solvent delivery pump coupled to a 20 μl Rheodyne 7125 manual injector, a model 484 variable wavelength UV detector and a model 741 Data Module printer. A Nova-Pak C_{18} Guard-Pak as precolumn and a stainless steel Nova-Pak C_{18} column (4 μm, 15 $\times$ 0.39 cm I.D.) served for chromatographic separations, performed at room temperature. The mobile phase: acetonitrile/phosphoric acid (20 mM, pH 2.3) (15:85, v/v) + 2.5 mM Na-HSA was vacuum filtered with a Waters Associates (Milford, MA, USA) filtering kit, Ø = 0.45 μm. Helium was used for degassing before pumping of the mobile phase at 1.5 ml/min, and the stabilisation period at the beginning of the runs was 30 min. Detection was at 278 nm. All the runs were performed with duplicate injections.

Calibration curves were constructed with ciprofloxacin concentrations 5, 10, 15, 20, 30, 40 and 50 μg/ml on 12 separate days. The intra-day repeatability of the chromatographic system was determined with six replicate injections of a solution containing 15 μg/ml of ciprofloxacin. This same solution was used during three months to monitor the inter-day reproducibility. The accu-

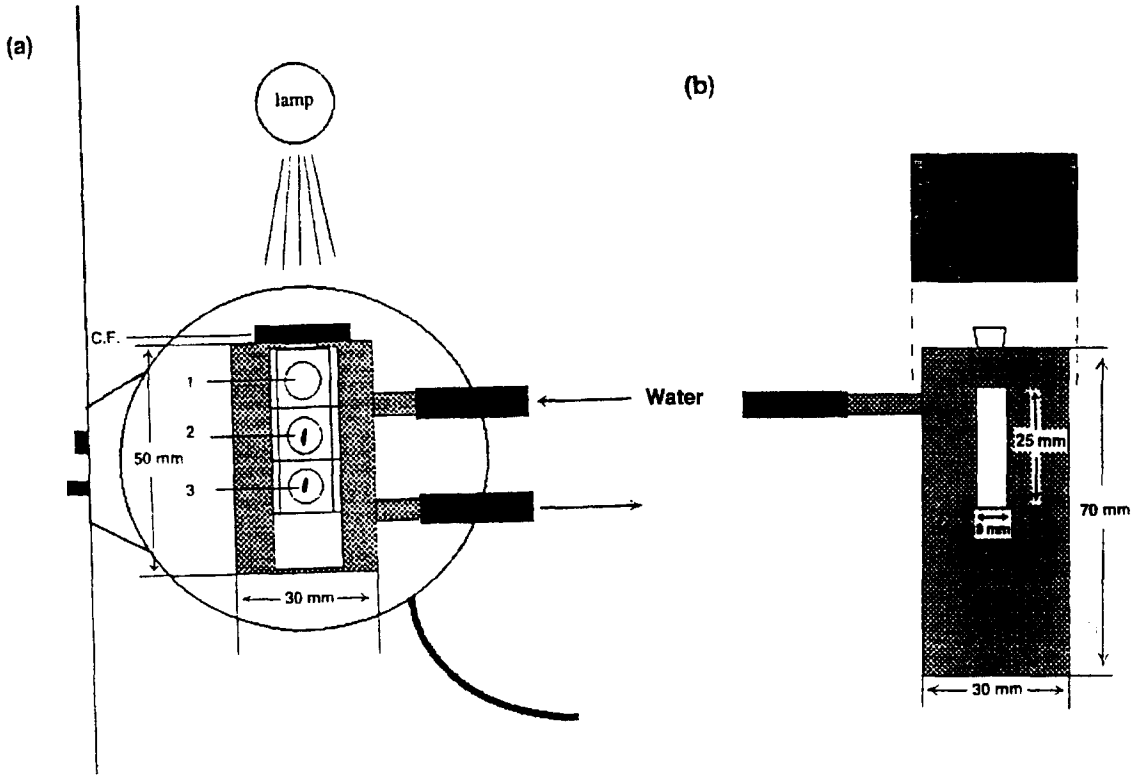

Fig. 2. Schematic representation of the irradiation apparatus (a) from above and (b) from the front. Detail identification: Corning filter CS-7-54 (C.F.), potassium chromate solution (5×10^{-4} M) cuvette (1), sample cuvette (2), iron(III) oxalate solution (6×10^{-3} M) cuvette (3) (Ulvi, 1995, with permission from Elsevier Science B.V. Amsterdam Publishing Division).

racy of the method was determined with six separate ciprofloxacin hydrochloride solutions (5×10^{-5} M) diluted from individual stock solutions (5×10^{-3} M). Standard solutions were co-chromatographed daily to check that the calibration was working.

Other Waters' equipment was used for the peak purity control: two 501 pumps coupled to an automated gradient controller with the same kind of injector as mentioned above, a model 991 diode-array detector with NEC PowerMate 386/25 computer, and photodiode array (PDA) software combined with a 5200 printer/plotter. The UV spectra were recorded in the range 210–350 nm. The precolumn, column and the mobile phase were the same as for the first-mentioned apparatus.

2.3. Photodegradation of ciprofloxacin

A high-pressure mercury lamp TQ 718, equipped with a quartz glass cooling mantle (Hanau, Germany), served as radiation source. Acetic acid solution (10%) was used at night to clean the mantle. The detailed technical procedure for the cuvette stand with magnetic stirring and water-cooling has been described by Ulvi (Ulvi, 1995) (Fig. 2). A Corning CS-7-54 filter together with the potassium chromate (5×10^{-4} M) solution in cuvette 1 (Fig. 2a) was used to isolate the wavelength 313 nm region.

The investigations at different pH were performed with the following ciprofloxacin hydrochloride (5×10^{-5} M) buffer solutions (Diem and Lentner, 1970): citrate (Sørensen) pH 3.0, 4.0,

5.0 and 6.0, acetate (Walpole) pH 5.0, phosphate (Sørensen) pH 5.0 and borate (Sørensen) pH 8.6 and 10.6.

The effect of concentration on the photodegradation of ciprofloxacin hydrochloride was quantitatively monitored with solutions 5×10^{-5} M, 2.5×10^{-4} M and 5×10^{-4} M in citrate buffer pH 5.0 (Diem and Lentner, 1970).

Aliquots of 3.0 ml were irradiated in 1-cm (I.D.) glass cuvettes (cuvette 2 in Fig. 2a) with the lamp TQ 718 at 500 W after 15 min stabilisation period. The distance between the lamp and the Corning filter was 5.0 cm. Before the HPLC runs, ciprofloxacin hydrochloride solutions with initial concentration of 5×10^{-5} M were filtered, as is, through Acrodisc LC 25, Ø 22 μm (Gelman Instrument Company, Ann Arbor, Michigan, USA). Solutions with initial concentrations of 2.5×10^{-4} M and 5×10^{-4} M were diluted 1:5 and 1:10, respectively, before filtration. The maximum follow-up time for the photodegradation was 150 min and all experiments were made at least in duplicate. Reference samples with each concentration and appropriate solvents were prepared for all determinations. These references also served as dark controls. No decomposition occurred during one month, indicating the observed degradation to be light-induced. The sample vials were covered with aluminium foil after preparation for HPLC.

The repeatability of the photodegradation procedure was tested in six successive irradiations of ciprofloxacin (5×10^{-5} M) hydrochloride in citrate buffer pH 5.0 with stressing periods of 15 and 60 min.

Three concentrations of ciprofloxacin (5×10^{-5} M, 5×10^{-4} M and 5×10^{-3} M) hydrochloride in citrate buffer pH 5.0 were exposed to daylight for 15 days in March on a west-facing window-sill. A parallel procedure was performed with the same three concentrations diluted from Ciproxin™ infusion fluid with 0.9% saline solution containing 0.11 mg/ml lactic acid (pH 3.5). Samples of 0.5 ml were taken from 10-ml glass vials at appropriate intervals and prepared for HPLC quantification as described above.

2.4. Irradiation intensity and reaction quantum yield

The intensity of the high-pressure mercury arc lamp and the amount of light absorbed by the samples were measured by ferrioxalate chemical actinometry. Preparation of ferrioxalate followed the instructions published by Hatchard and Parker (1956) and the procedure in actinometric measurements was according to Kuhn et al. (1989).

For the measurement of the amount of irradiation passing into the reaction vessel, two cuvettes (1 and 3 in Fig. 2a) were placed one behind the other and exposed to radiation for 1.5 to 6 min. These measurements were repeated at the beginning and end of every working day. Two successive determinations with all three cuvettes (Fig. 2a) were needed to measure the amount of light absorbed by the sample. In the experiments, the sample cuvette 2 (Fig. 2a) contained, alternately, pure solvent and ciprofloxacin hydrochloride solution. Stressing periods for pure solvents and sample solutions varied from 3 to 6 and from 3 to 11 min, respectively. One milliliter of reduced actinometric solution was introduced into a 10-ml volumetric flask containing a pre-mixed solution of 0.1% *o*-phenanthroline solution (in water) and acetate buffer (4 ml + 0.5 ml). Water was used to adjust the final volume. After 30 min in the dark, the absorbance of the Fe(II) ion/*o*-phenanthroline complex that formed was measured at 510 nm against a blank.

The photon flows per unit volume and per unit time were calculated according to Kuhn et al. (1989) and the quantum yields of reactions according to Moore (1987).

3. Results and discussion

3.1. HPLC method

The HPLC method (Torniainen and Mäki, 1995) developed for monitoring the photodegradation of ciprofloxacin was initially carried out without a precolumn and was validated on sample concentrations ten times higher than in these stud-

ies. Reliability with the micromolar solutions and with use of a precolumn was very good, however.

Calibration graphs (n = 12), where peak area was plotted against concentration, were linear with correlation coefficients $r > 0.9999$. The mean intercept was -2.385×10^4 and the mean slope = 9.660×10^7; 95% confidence limits for the intercept were from $+0.468 \times 10^4$ to -5.238×10^4. Corresponding confidence limits for the slope were from 9.5607×10^7 to 9.7598×10^7. The intra-day repeatability of the peak areas (15 μg/ml) and retention times was good: relative standard deviation (R.S.D.) values ≤ 0.7% (n = 6). The inter-day precision gave an R.S.D. of 1.1% (n = 12) for peak areas and R.S.D. of 1.7% (n = 12) for retention times during a working period of 3 months. The accuracy expressed as percentage of the nominal concentration was 98–101% (R.S.D. = 1.7%, n = 6).

3.2. Photodegradation

Photochemical studies on ciprofloxacin hydrochloride were carried out in the wavelength region around 313 nm, where the drug has a second absorption maximum with low intensity. The wavelength was isolated from the mercury lamp with a Corning CS-7-54 filter — potassium chromate combination. Irradiation intensity was measured using ferrioxalate chemical actinometry, which is based on light-induced reduction of Fe(III) and subsequent determination of Fe(II) ions by colorimetry with o-phenanthroline (Kuhn et al., 1989). During 3-months working period, the intensity of the high-pressure mercury lamp was $0.95 \pm 0.05 \times 10^{16}$ photons $\times$ s^{-1} into 3 ml of actinometric solution when the path length was 1 cm (n = 90). Daily cleaning of the lamp sleeve with acetic acid was essential to keep the light intensity stable.

The repeatability of the photodegradation was tested with a ciprofloxacin hydrochloride solution (5×10^{-5} M) in citrate buffer pH 5.0. In six replicate 15 min irradiations, an R.S.D. of 1.8% was achieved for the concentration of the parent compound remaining, and in 60 min irradiations the R.S.D. was 2.9% (n = 6).

Table 1
The quantum yields (Φ) of photodegradation reactions of ciprofloxacin (initial concentration 5×10^{-5} M)

pH	Φ
3.0	0.0015
4.0	0.0016
5.0	0.0029
6.0	0.023
8.6	0.048
10.6	0.016

$$\Phi = \frac{\text{number of molecules reacted per unit volume and unit time}}{\text{number of photons absorbed per unit volume and unit time}}$$

3.3. Effect of pH and buffer type

A pH-dependence of the photodegradation rate has been reported in the literature, e.g. for furosemide (Bundgaard et al., 1988) and sulphamethoxazole (Zhou and Moore, 1994). Photodegradation of ciprofloxacin was examined in buffer solutions in the pH range 3.0 to 10.6.

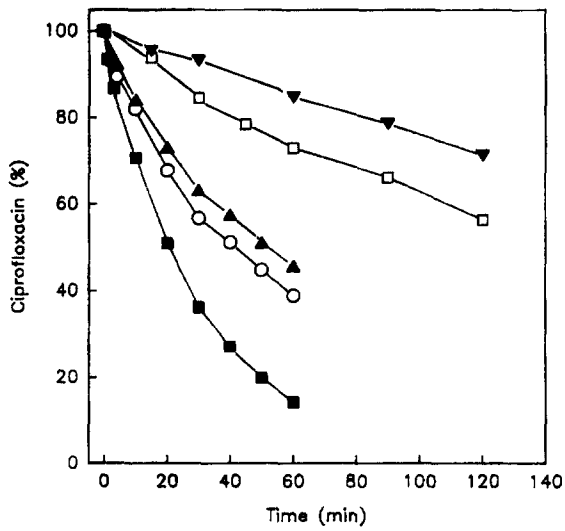

Fig. 3. The time course for the photodegradation of ciprofloxacin hydrochloride (initial concentration 5×10^{-5} M) in buffer solutions: citrate pH 4.0 (▼), pH 5.0 (□), pH 6.0 (○); borate pH 8.6 (■), pH 10.6 (▲). Radiation source: mercury lamp TQ 718.

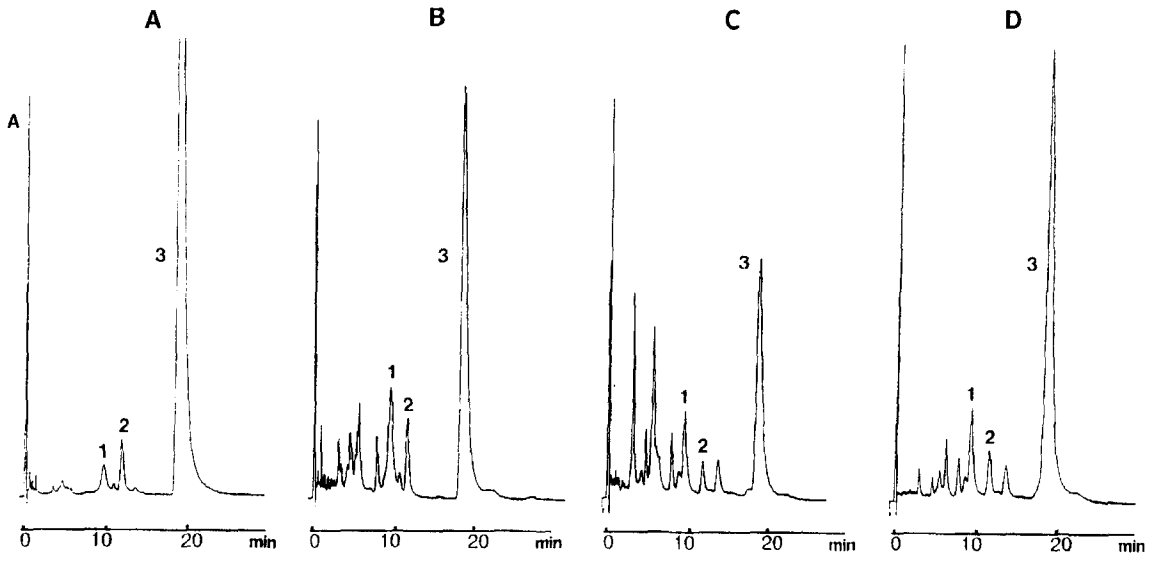

Fig. 4. Chromatograms of ciprofloxacin hydrochloride solutions exposed to mercury lamp TQ 718 for 60 min: citrate buffer pH 4.0 (A), pH 6.0 (B); borate buffer pH 8.6 (C), pH 10.6 (D). LC conditions: precolumn, Nova-Pak C_{18} Guard-Pak; column, Nova-Pak C_{18} (4 μm, 15 × 0.39 cm I.D.); mobile phase, acetonitrile/phosphoric acid (20 mM, pH 2.3) (15:85, v/v) containing 2.5 mM 1-heptanesulphonic acid sodium salt; flow rate, 1.5 ml/min; detection, UV at 278 nm. Peak identification: compound II = 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid (1), compound I (2), ciprofloxacin (3).

The quantum yields for the photochemical reaction were calculated after about 10% degradation, in a linear part of the reaction. The data in Table 1 and Fig. 3 demonstrate clearly the influence of the pH on the degradation. After 1 h exposure to radiation, the photodegradation increased from 15% loss of the parent compound at pH 3.0 and pH 4.0 towards the maximum at pH 8.6, where only 15% of ciprofloxacin was left (Fig. 3). At pH 10.6 the rate decreased near to the level at pH 6.0, resulting in 55% loss of ciprofloxacin after 1 h stressing with the mercury lamp. Ciprofloxacin is an ampholytic compound with pK_a values of 6.09 for the carboxylic group and 8.74 for the nitrogen on the piperazinyl ring (Ross and Riley, 1990). The isoelectric point of the zwitterion is at pH 7.4. Ciprofloxacin seemed to be most sensitive to photodegradation in zwitterionic form at slightly basic pH. The maximum stability of the drug was observed in solutions at pH 3.0 to 4.0, where the COOH group is not ionized and the basic nitrogen completely protonated. From the practical point of view, the stability of ciprofloxacin in acidic milieu is important because the pH of liquid pharmaceutical formulations varies between 3.5 and 5.5 (US Pharmacopeia/National Formulary 23/NF 18, 1995).

Besides the reaction rate, the pH of the solution also affected product formation (Fig. 4). In acidic solutions two major products were formed, which were designated compounds I and II in a previous work (Torniainen and Mäki, 1995). Compound II has been isolated and identified as 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid (Askolin et al., 1995). Detailed structure elucidation by various spectrometric methods will be reported in a separate article. Several additional degradation products were detected with increasing pH. In more basic solutions, secondary degradation appeared to occur on longer exposure to radiation. The UV spectra of the degradation products recorded from the HPLC run after 30 min irradiation at pH 8.6 were very similar to the spectrum of ciprofloxacin, indicating an unchanged chromophoric system in these products (Fig. 5).

Effect of buffer species on the degradation of ciprofloxacin was studied at pH 5.0 using acetate,

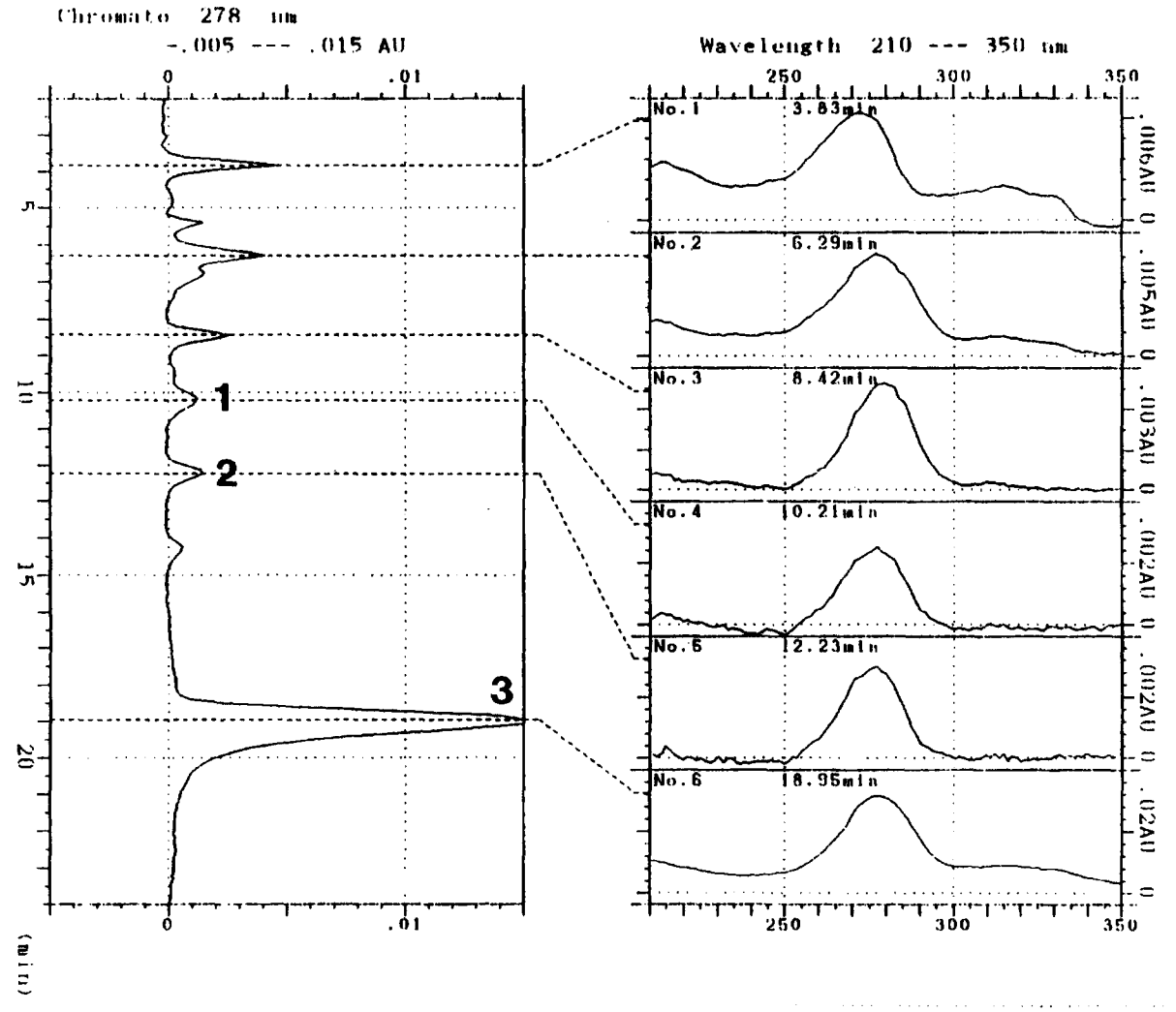

Fig. 5. Chromatogram of ciprofloxacin hydrochloride in borate buffer pH 8.6 exposed to mercury lamp TQ 718 for 30 min, and UV spectra of ciprofloxacin and its degradation products. LC conditions: photodiode array detector. Other conditions and peak identification as in Fig. 4.

citrate and phosphate buffers. The reaction rates and product profiles were approximately the same in all three solutions indicating that the buffer type had no substantial influence on the degradation.

3.4. Effect of concentration

The photodegradation of ciprofloxacin was followed in citrate buffer pH 5.0 on three concentration levels (Fig. 6). The reaction rate was inversely proportional to the initial drug concentration. The half-lives calculated according to first-order kinetics were 1.7, 7.6 and 15 h with ciprofloxacin hydrochloride concentrations 5×10^{-5} M, 2.5×10^{-4} M and 5×10^{-4} M, respectively. Drug content in commercial infusion fluid is about 10 times higher than the highest concentration used in the present study, and the tendency to photodegradation would presumably be correspondingly less.

A similar concentration-dependent rate of photodegradation was observed in solutions exposed to daylight ($\lambda > 300$ nm) (Table 2). The

Table 2
Degradation of ciprofloxacin hydrochloride in citrate buffer pH 5.0 and Ciproxin™ (ciprofloxacin lactate) infusion fluid dilutions exposed to daylight for 15 days in March 1995 in Finland

Initial concentration of ciprofloxacin	Concentration of ciprofloxacin remaining in citrate buffer pH 5.0	Concentration of ciprofloxacin remaining in Ciproxin™ infusion dilution	
	(%)	(%)	pH
5×10^{-5} M	84.3	94.7	3.5
5×10^{-4} M	94.5	98.0	3.7
5×10^{-3} M	98.0	98.1	3.9

highest concentration level (5×10^{-3} M) equalled the commercial infusion fluid and only 2% decrease in ciprofloxacin content was observed after 2 weeks stressing period. Accelerated photodegradation was observed in 1:100 dilutions of the same solutions. The higher reaction rate in citrate buffer pH 5.0 than in dilutions of Ciproxin™ infusion fluid, was due in part to the 1.5 unit higher pH.

4. Conclusions

The results obtained in photochemical studies

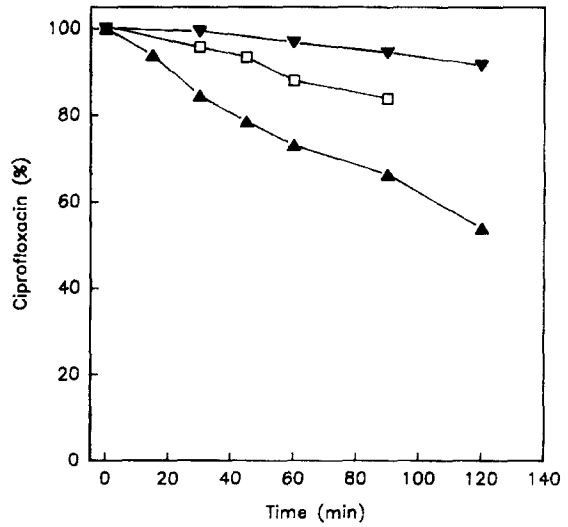

Fig. 6. Effect of drug concentration on the photodegradation of ciprofloxacin hydrochloride in citrate pH 5.0 buffer. (▲) 5×10^{-5} M, (□) 2.5×10^{-4} M, (▼) 5×10^{-4} M. Radiation source: mercury lamp TQ 718.

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Pon ciprofloxacin show that the degradation is strongly dependent on the pH of the solutions and the initial drug concentration. Ciprofloxacin and liquid pharmaceutical formulations of it should be protected from light during storage, but special arrangements for the handling of liquid dosage forms of ciprofloxacin in hospital practice would seem unnecessary.

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