ELSEVIER

Journal of Chromatography A, 837 (1999) 253–265

JOURNAL OF CHROMATOGRAPHY A

Development of capillary electrophoresis methods for the analysis of fluoroquinolones and application to the study of the influence of humic substances on their photodegradation in aqueous phase

Ph. Schmitt-Kopplin[a,*], J. Burhenne[b], D. Freitag[a], M. Spiteller[b], A. Kettrup[a]

[a]*GSF–National Research Center for Environment and Health, Institute of Ecological Chemistry, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany*

[b]*University of Kassel, Department of Ecological Chemistry and Ecotoxicology, Nordbahnhofstrasse 1a, D-37213 Witzenhausen, Germany*

Received 8 May 1998; received in revised form 9 December 1998; accepted 16 December 1998

Abstract

Analytical techniques in capillary zone electrophoresis (CZE) and capillary electrokinetic chromatography (MEKC) were developed for the analysis of fluoroquinolone carboxylic acids (norfloxacin, ciprofloxacin, ofloxacin, enrofloxacin, danofloxacin) and their major degradation products. The theoretical determination of the charge densities of the studied compounds allowed the rapid development of the separation buffer conditions. These rapid estimations can be used as an effective screening tool in capillary electrophoresis (CE) method development. The two CE methods were applied to follow the photostability of enrofloxacin with and without humic substances under natural sunlight conditions. Enrofloxacin showed an average half-life of 2.0 h under summer sunlight conditions and the photolysis kinetic decreased in the presence of humic acids. The presence of humic substances in irradiated solution caused changes in the measured photodegradation product profile. Studies in affinity capillary electrophoresis (ACE) of enrofloxacin and its degradation products with the dissolved humic acids showed a lower adsorption potential of enrofloxacin to the humic phase than the degradation products. The adsorption of some photodegradation products to the dissolved humic matrix may explain the differences in the measured photodegradation products concentration in irradiated solutions. ACE turned out to be a rapid screening tool for the comparison of the adsorption potential of active ingredients and their degradation products to dissolved organic phases using very small amounts of sample.

Keywords: Affinity capillary electrophoresis; Photodegradation; Fluoroquinolones; Quinolones; Humic acids

1. Introduction

Quinolones (also known as fluoroquinolones, quinolone carboxylic acids or 4-quinolones) are chemotherapeutic agents with antibacterial activity and belong to the family of gyrase inhibitors. They show striking potency against enteric Gram-negative bacilli, lesser activity against nonenteric Gram-negative bacilli and staphylococci, and generally marginal activity against streptococci and anaerobes [1]. Quinolones are used in human medicine as well as in veterinary medicine, especially in the field of animal

*Corresponding author. Corresponding address: GSF–National Research Center for Environment and Health, Schulstrasse 10, D-85356 Freising–Attaching, Germany. Tel: +49-8161-987-720; fax: +49-8161-81612.

E-mail address: SCHMITTPH@GSF.de (Ph. Schmitt-Kopplin)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00079-5

breeding. Considerable amounts of quinolones are applied as antibacterial agents in large-scale husbandry, above all in case of chickens, cows and pigs [2–4]. The elimination of the active ingredient occurs mainly as parent compound [3,5]. Important quantities are transported to agricultural areas by means of liquid manure. Therefore the sorption behavior and binding mechanism of selected quinolones to different soils and clay minerals were studied in laboratory experiments. At least 90% of the applied quinolone were sorbed onto the soils [6]. Additionally, the photolytic degradation of ^{14}C-labeled enrofloxacin was studied in aqueous solution using a sunlight simulating xenon lamp. During 18 days more than 15% of the radioactivity were mineralized by forming $^{14}CO_2$. The main photodegradation products were identified. They were similar to the degradation products formed from ciprofloxacin, norfloxacin and danofloxacin [7–9]. The photolysis of levofloxacin was reported by a Japanese group. They found degradation products mainly with alterations at the piperazine substitution [10].

The analysis of quinolones is dominated by high-performance liquid chromatography (HPLC) and UV detection. For residue analysis, the application of fluorescence detection is recommended. The separation can be performed with RP-18 columns using water and acetonitrile as mobile phases [11]. The analysis via gas chromatography (GC) is possible after methylation of the carboxylic acid group. The application of capillary electrophoresis (CE) techniques for the determination of ciprofloxacin and its metabolite desethyleneciprofloxacin has been published recently [12]. The application of CE to the separation of different quinolones and their main photodegradation products is shown in the present study.

CE with its various modes of operation (capillary zone electrophoresis – CZE, micellar electrokinetic capillary chromatography – MEKC) has proven to be useful in the analysis of pesticides [13–16]. The concentration limit of detection in CE with UV detection is relatively high, thus limiting its use in the determination of pesticides in trace levels. Only the use of laser-induced fluorescence (LIF) detection or the combination of on-line and off-line concentration procedures can solve these problems of detectability and allow CE to become one of the most suitable techniques for the separation and determination of pesticides and their degradation products in water and soil samples [17]. Affinity capillary electrophoresis (ACE) is nowadays a well proven method for the study of molecular interaction. A recent review [18] showed that this method has a broad application potential, since the only conditions that have to be satisfied are that (i) the unbound molecules have a mobility different from the complex, (ii) one of the reactants can be detected and quantified and (iii) the kinetics of association and dissociation are sufficiently fast compared to the time scale of the separation. The use of ACE to determine simultaneously the partitioning of different cationic *s*-triazines between water and dissolved humic substances was demonstrated by the authors elsewhere [19].

The aim of this study was (i) to develop simple and efficient CE methods for the separation and quantitative determination of quinolones and their main photodegradation products; (ii) to describe quantitatively with CZE and MEKC the photolysis of enrofloxacin in the presence of dissolved humic acids and (iii) to evaluate in a rapid screening with ACE the partitioning of the degradation products between water and the dissolved humic acids.

2. Materials and methods

2.1. Instrumentation

CE instrumentation consisted of a Beckman P/ACE 2050 Series HPCE with Beckman System Gold Chromatography Software. Uncoated fused-silica CE columns [57 cm (length to the detector 50 cm)×75 μm I.D.×375 μm O.D.] were obtained from Polymicro (Laser 2000, Wessling, Germany). The UV detection wavelength was 280 nm and LIF detection was achieved with a helium–cadmium laser series 74 at an excitation wavelength of 325 nm, emission wavelength 420 nm, obtained from Laser 2000.

2.2. Buffers

CZE and MEKC buffers were prepared by mixing 0.1 *M* $NaHCO_3$ and 0.1 *M* Na_2CO_3 in amounts to reach the desired pH, and then diluting the solution

with an equal volume of distilled water or aqueous sodium dodecyl sulfate (SDS) (200 m*M*), respectively.

2.3. Separation

CE conditions: temperature, 30°C; voltage, 20 kV; the anodic injection was in the hydrodynamic mode for 5 s or 10 s.

2.4. Chemicals

Enrofloxacin [1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid, Bay VP 2674], ciprofloxacin (1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid, Bay O 9867), norfloxacin (1-ethyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, AB-2203), ofloxacin {9-fluoro-2,3-dihydro-3-methy-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid} and danofloxacin {1-cyclopropyl-6-fluoro-7-(1*S*,4*S*-5-methyl-2.5-diazabicyclo[2.2.1]heptan-2yl)1,4-dihydro-4-oxoquinoline-3-carboxylic acid, PC 3909} are the parent molecules; ciprofloxacin, decarboxylated enrofloxacin, Nos. 5, 7, 8, decarboxylated Nos. 8, 11 and 12 are degradation products of enrofloxacin as reported Burhenne and co-workers [7,8].

Enrofloxacin, ciprofloxacin as well as decarboxylated enrofloxacin, compounds Nos. 5, 7, 8 and decarboxylated No. 8 were received from Bayer (Leverkusen, Germany). Norfloxacin was obtained from MSD Pharma and danofloxacin from Pfizer. Compounds 11 and 12 were isolated by preparative HPLC as described elsewhere [7,8]. Compounds enrofloxacin decarboxalated and No. 8 decarboxylated are the decarboxylated forms of No. 8 and enrofloxacin; they were not considered as relevant photodegradation products of enrofloxacin in previous studies under Xenon lamp irradiation. The structures of the studied compounds are given in Table 1.

2.5. Procedures

Molecular modeling was performed using the software Alchemy 2000 (Tripos, St. Louis, MO, USA). The corresponding pK_a and log K_{ow} (octanol–water coefficient) values were estimated with the Pallas 1.1, Pkalc 3.0 and PrologP for Windows (CompuDrug Chemistry, Budapest, Hungary). All calculated values are given in Table 1. Table 1 also gives the total charge of the analytes at pH 9.2 and the distribution in the molecule in negative and positive charges.

Photodegradation was carried out in quartz vials on 14 July 1997 under natural sunlight outdoor conditions (average irradiation intensity 500 W/m^2) at a temperature between 20°C and 25°C. The irradiation intensities and the temperatures in dependency of the time are shown in Fig. 1. The fluctuations in the intensities can be attributed to the alternation of tree shade and clouds, especially in the afternoon. From these results it can be deduced that natural conditions are far away from adjusted simulated sunlight conditions as used by Schmitt et al. [16] and Burhenne et al. [7]. Three solutions containing 5 mg/l of enrofloxacin in distilled water were exposed simultaneously to sunlight. One solution contained only the biocide. The second solution additionally contained 20 mg/l fulvic acids and the third solution 20 mg/l humic acids. The fulvic and humic acids were extracted with the IHSS standard extraction procedure (NaOH extraction followed by HCl precipitation and XAD8 resin concentration of the fulvic acids) from a cultivated loamy brown soil [''Forschungsverbund Agrarökosystem München'' (FAM) in Scheyern, Germany] and were described elsewhere [16,20]. Aliquots were taken at different times and analyzed with CZE and MEKC to follow the degradation of enrofloxacin and the evolution of photoproducts. Blank solutions were kept in the dark at 4°C and analyzed to certify that the decreasing concentration of enrofloxacin was only caused by photochemical processes.

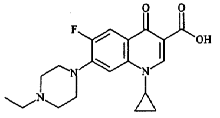
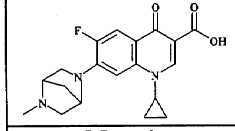
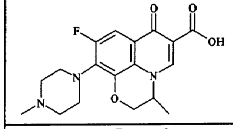
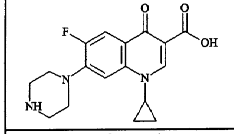
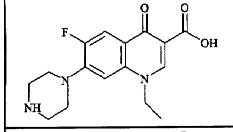
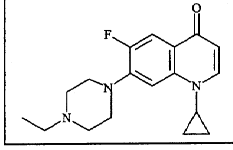
3. Results and discussion

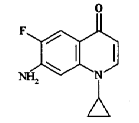
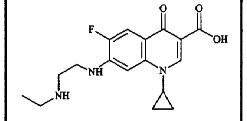
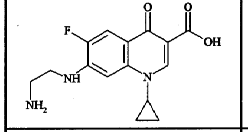
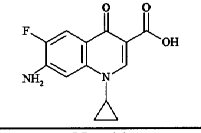
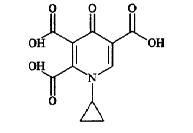
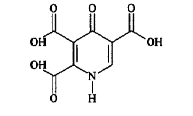
3.1. Separation method development with capillary electrophoresis:

3.1.1. Capillary zone electrophoresis

The migration time does not reflect the effective electrophoretic mobility, μ, of the analyte in the separation system which is independent of the electroosmotic flow (EOF). The effective electrophoretic

Table 1
Structures of the studied quinolones and their calculated physico–chemical parameters

Studied quinolones and metabolites	total charge at pH 9.2 positive / negative charge (pH 9.2) pK_a-values	molecular weight molar volume molar surface log K_{ow}
Enrofloxacin 	-0.97 +0.03 / -1.0 *pK_a 6.21 (-COOH)* *pK_a 7.74 (=NH^+)*	359.4 g/mol 311.6 cm³/mol 362.3 cm²/mol 2.13
Danofloxacin 	-0.92 +0.08 / -1.0 *pK_a 6.21 (-COOH)* *pK_a 8.14 (=NH^+)*	359.4 g/mol 300.7 cm³/mol 345.7 cm²/mol 1.78
Ofloxacin 	-0.97 +0.03 / -1.0 *pK_a 5.97 (-COOH)* *pK_a 7.65 (=NH^+)*	361.4 g/mol 298.6 cm³/mol 340.0 cm²/mol 1.52
Ciprofloxacin 	-0.68 +0.31 / -1.0 *pK_a 6.27 (-COOH)* *pK_a 8.87 (=NH2+)*	331.4 g/mol 277.5 cm³/mol 324.0 cm²/mol 1.35
Norfloxacin 	-0.69 +0.31 / -1.0 *pK_a 6.26 (-COOH)* *pK_a 8.85 (=NH_2^+)*	319.4 g/mol 269.1 cm³/mol 319.0 cm²/mol 1.25
decarboxylated Enrofloxacin 	0.03 +0.03 / -0.0 *pK_a 7.74 (=NH^+)*	315.4 g/mol 284.50 cm³/mol 333.00 cm²/mol 3.06

Studied quinolones and metabolites	total charge at pH 9.2 positive / negative charge (pH 9.2) pK_a -values	molecular weight molar volume molar surface log K_{ow}
decarboxylated No. 8 	0.0 +0.0 / -0.0	218.2 g/mol 182.90 cm³/mol 220.86 cm²/mol 1.74
No. 5 	-0.02 +0.98 / -1.0 *pK_a 6.20 (-COOH)* *pK_a 10.93 (=NH_2^+)*	333.4 g/mol 289.0 cm³/mol 345.4 cm²/mol 1.38
No. 7 	-0.09 +0.91 / -1.0 *pK_a 6.17 (-COOH)* *pK_a 10.18 (-NH_3^+)*	305.3 255.3 cm³/mol 304.8 cm²/mol 0.60
No. 8 	-1.00 +0.0 / -1.0 *pK_a 6.20 (-COOH)*	262.2 209.5 cm³/mol 250.1 cm²/mol 0.80
No. 11 	-3.00 +0.0 / -3.0 *pK_a 6.33 (-COOH)* *pK_a 6.41 (-COOH)* *pK_a < 2.0 (-COOH)*	267.2 201.0 cm³/mol 232.9 cm²/mol -0.45
No. 12 	-3.00 +0.0 / -3.0 *pK_a 4.59 (-COOH)* *pK_a 5.33 (-COOH)* *pK_a < 2.0 (-COOH)* *pK_a 6.31 (=NH_2^+)*	227.1 161.2 cm³/mol 193.0 cm²/mol -1.26

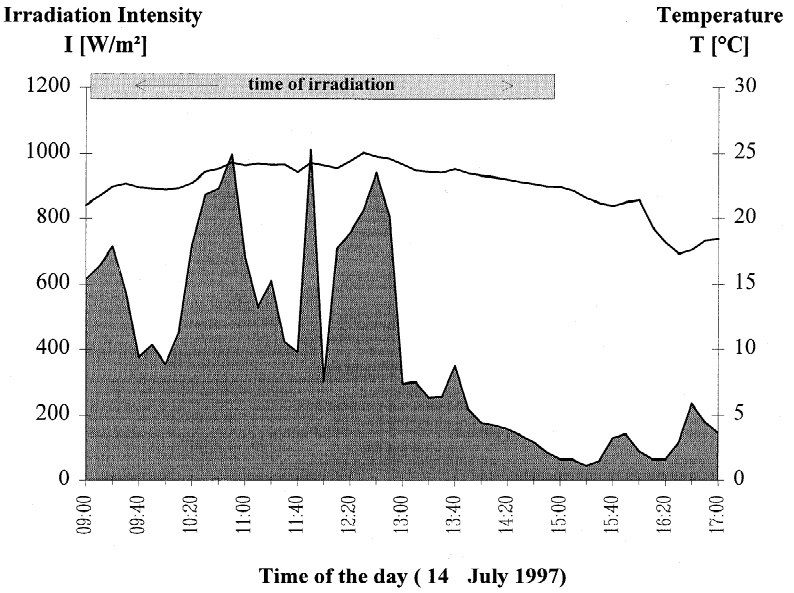


Fig. 1. Behavior of the temperature and the irradiation intensities during the photodegradation of enrofloxacin under natural sunlight conditions.

mobilities (μ) of the analytes are calculated by subtracting the electroosmotic flow (μ_{eof}) from the measured electrophoretic mobilities (μ_{mes}) – EOF correction – and are used as the absolute electrophoretic values when comparing separations and calculating the binding constants.

The effective mobility μ is a fundamental parameter that can be approximated from Stokes' law [21]:

$$\mu = \frac{q}{6\pi\eta R} \quad (1)$$

where q is the net charge, R the Stokes radius, and η the viscosity.

Semi-empirical parameters, already described in the 1960s [22] to predict mobilities of peptides in electrophoretic separation systems, were adapted to CE separation of polypeptides and proteins: the effective mobility μ can be described with a charge-to-size parameter where the size of the molecules are approximated by their molecular mass M. The mobility was found to be a continuous function of $M^{-1/3}$ to $M^{-2/3}$, depending on the magnitude of M and the ionic strength of the buffer [23]. Since a good correlation can be found between the mobilities and the charge densities of the studied analytes, both charge per molar volumes and charge per molar surfaces (related to the Stokes radius R) could also be involved in the formula.

Knowing the dissociation constants (pK_a), the theoretical charge Z of small molecules can be calculated at each pH. Experimental and theoretical values were not found in the literature for all studied compounds, therefore we calculated the dissociation constants with an appropriately validated software [24]; the molar volumes V_m and molar surfaces S_m were also obtained from computer calculations (Alchemy 2000). All the calculated data are resumed in Table 1: the given pK_a corresponds to acidity of the carboxyl group of the nitrogen located at the distant end of the piperazine [25]. There is a very good accordance between the theoretical dissociation constants calculated for enrofloxacin ($pK_a = 6.21$ and $pK_a = 7.74$) and the data obtained by potentiometric titration ($pK_a = 6.27$, $pK_a = 7.73$) [6]; the same accordance was found for norfloxacin [25]. The structural fragments used for the calculation of the dissociation constants of the degradation products are very similar to enrofloxacin, therefore the calculated values for the degradation products are expected to be very close to the real dissociation constants.

Fig. 2 illustrates the variation in calculated $Z/M^{2/3}$ for the five quinolones and their main degradation

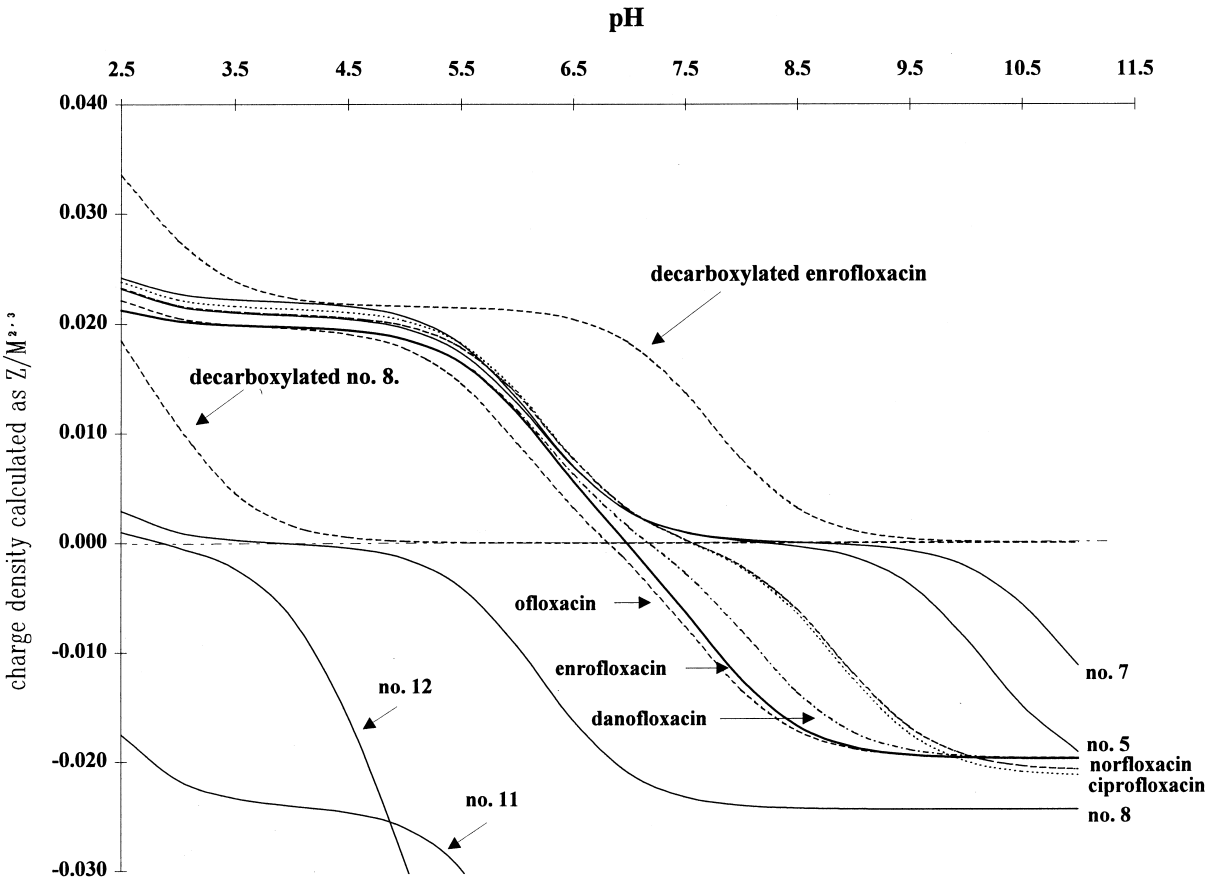


Fig. 2. Theoretical determination of the charge densities ($Z/M^{2/3}$) of the studied quinolones as a function of the pH.

products as a function of the pH. At low pH values only compounds 8, 11 and 12 are expected to be found as anions; all others are positively charged. The best separation based on this parameter is expected to be found at a pH between 9.0 and 9.5, where all compounds would be analyzed as anions. When representing the variations in Z/V_m or Z/S_m versus pH, the analysis leads to the same conclusions, with the only difference that ciprofloxacin and norfloxacin take higher charge densities than enrofloxacin, ofloxacin and danofloxacin already at lower pH (around 9.0).

CZE separation trials with the five parent quinolones resulted mainly in comigration of the compounds as predicted by the parameter; best separation with a 50 m*M* carbonate buffer at pH 9.2 showed three comigrating compounds (danofloxacin, enrofloxacin and ofloxacin) separated from ciprofloxacin and norfloxacin (electropherogram not shown). The experimental effective mobilities of enrofloxacin and its degradation products (ciprofloxacin, Nos. 5, 7, 8, decarboxylated enrofloxacin, decarboxylated No. 8) were plotted against the theoretical $Z/M^{2/3}$, Z/V_m and Z/S_m. The linear regression of the mobilities versus the theoretical charge densities gave correlation coefficients r^2 of 0.9817, 0.9946 and 0.9935 for the three plots, respectively. The corresponding electropherogram of the separation of enrofloxacin and its degradation products is given in Fig. 3a. The best correlation is found when using the molar volumes as description parameter; furthermore ciprofloxacin and norfloxacin

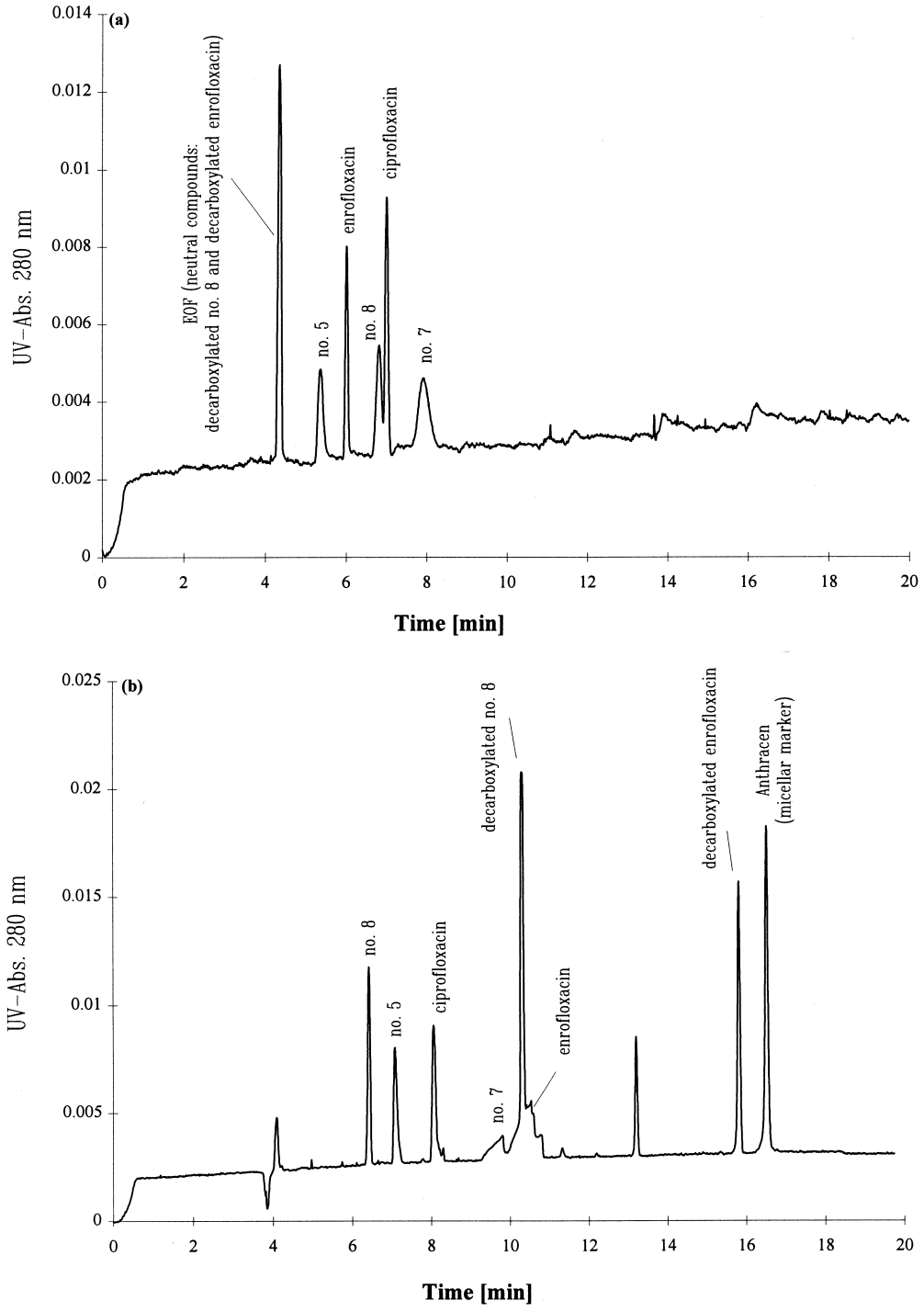


Fig. 3. (a) CZE separation of enrofloxacin and its degradation products (UV detection 280 nm; 20 kV, 30°C, 50 m*M* carbonate buffer, pH 9.2, sample concentration 5 mg/l). (b) MEKC separation of enrofloxacin and its degradation products (UV detection 280 nm; 20 kV, 30°C, 100 m*M* SDS, 25 m*M* carbonate buffer, pH 9.2, sample concentration 5 mg/l).

showed a higher effective mobility than enrofloxacin, ofloxacin and danofloxacin as predicted by the Z/S_m and the Z/V_m parameters.

The effective mobility of the anions shown in an electrophoretic run is always lower in absolute value than the EOF (if the mobility of the anions would be higher in magnitude than the EOF, the anions would migrate out of the capillary at the anode). The highest theoretical $Z/M^{2/3}$, Z/S_m or Z/V_m of anions that can thus be detected in the electropherogram can be calculated from the above mentioned linear regressions. Because the velocities of compounds 11 and 12 are calculated higher than the EOF, they will not be detected in this experimental configuration.

Some variations in the pH of the separation buffers from pH 5 to 12 resulted in peak coelutions as predicted with the parameters. These predictive parameters are very effective for the evaluation of the optimal separation pH conditions when designing the experiments. The $Z/M^{2/3}$ parameter is already good enough to get an adequate first approximation parameter of the separation potentials of a mixture when molar volume and surface data are not available.

3.1.2. Micellar electrokinetic chromatography

The electrophoretic separation in the MEKC mode is based on the relative distribution of the analytes between the aqueous phase and the micellar phase added to the separation buffer [26,27]. SDS is the most routinely used anionic detergent for the analysis of biocides with MEKC [28–30]. At a concentration over the critical micellar concentration (CMC), spontaneous aggregation of the surfactant molecules is caused by the increase of hydrophobic interactions. Hydrophobic interactions are less to be understood as attraction forces than rather as a passive way to diminish the degree of order of water-entropy increase [31]. These phenomena were well described for the formation of micelles and are also responsible for the formation and stability of biological membranes [32]. From this, the distribution of the analytes between the water and the micellar phase is based on the relative hydrophobicities of the analytes. The higher the log K_{ow} value of the analytes, the more these compounds are partitioned into the micelles. These interactions also include dipole–dipole, dipole–induced dipole interactions, and a recent study showed that, apart from a high dipolarity,

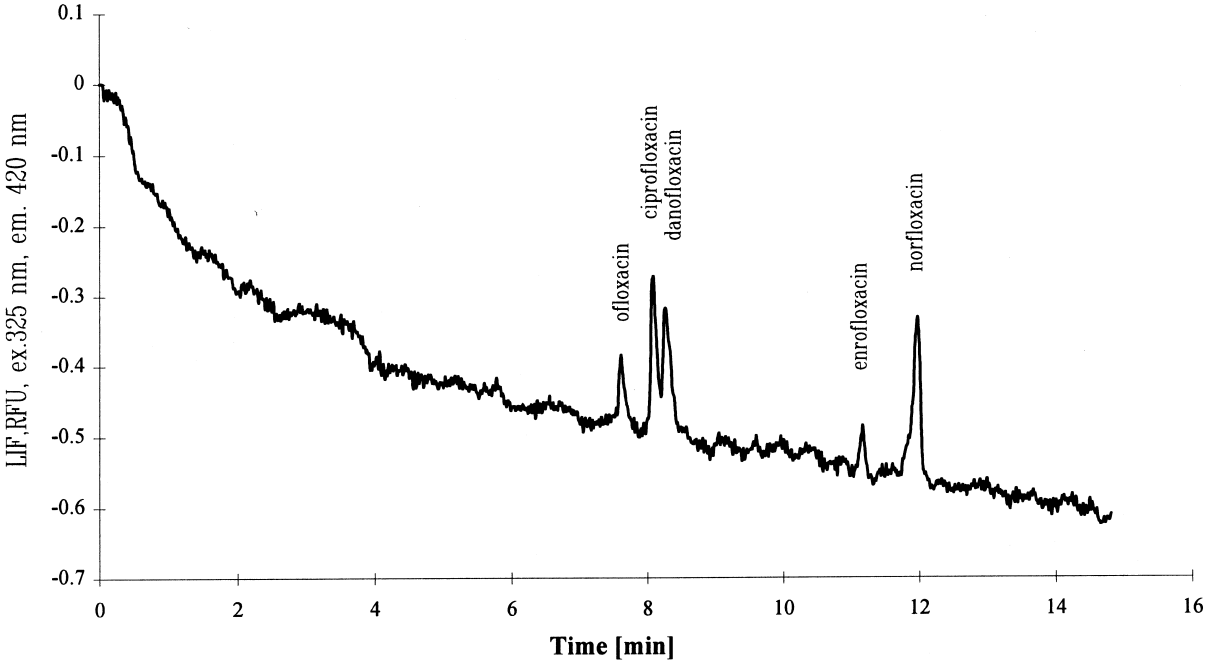


Fig. 4. MEKC separation of the five quinolones in a sample concentration of 0.6 μg/ml (LIF detection ex. 325 nm, em. 420 nm; 20 kV, 30°C, 100 m*M* SDS, 25 m*M* carbonate buffer, pH 9.2).

SDS micelles present strong hydrogen bond acidities [33].

The same buffer as in CZE (giving the highest difference in charge to size ratios between the studied analytes) was adjusted to 100 m*M* SDS at pH 9.2. Anthracene was taken as micellar marker and all compounds were separated under these conditions in an elution window from 4 to 17 min. The electropherograms of the MEKC separations of enrofloxacin and its photodegradation products are shown in Fig. 3b. Enrofloxacin coeluted with degradation product No. 8 decarboxalated. Fig. 4 shows the separation of the five parent quinolones under the same MEKC conditions.

Enrofloxacin, danofloxacin, ofloxacin and degradation product Nos. 8, 11 and 12 are negatively charged. Ciprofloxacin and norfloxacin present up to 1/3 positive charge from the end nitrogen of the piperazine ring. Nos. 5 and 7 possess one positive charge, decarboxylated enrofloxacin and decarboxylated No. 8 are neutral at pH 9.2 (Table 1).

The migration order of neutral compounds in MEKC is governed by their log K_{ow} (linear relation between the octanol–water partition coefficients and the capacity factors). The migration order of the quinolones in MEKC in Figs. 3b and 4 is not correlated to their hydrophobicities. Ciprofloxacin, norfloxacin and No. 7 show higher affinity to the anionic SDS micelles than expected from their log K_{ow} only. The migration order of these compounds is thus governed by a combined effect of their hydrophobicities (incorporation into the hydrophobic sites of the micelles) and their positive charge (ionic interactions between the negatively charged micelle surface and the cationic part of the analytes). Although No. 5 has a higher log K_{ow} and the same charge as No. 7, its affinity to SDS is lower (lower capacity factor). This can be explained by the ethyl substitution at the end nitrogen in No. 5. Positive charge is not directly available for the interaction and the ionic interaction of No. 5 with SDS is sterically hindered showing that the position of the charge has its importance.

3.2. Quality parameters for enrofloxacin quantification

The photodegradation of enrofloxacin was quantified by measuring with CZE the relative disappearance of enrofloxacin from the solution of known concentration (5 mg/l), so there was no need of its absolute quantification from solution. All quantifications were done with the same capillary and showed low variations in effective mobility (under 3%). Nevertheless, the linear range and the detection limit of the analytical method were measured. In CZE, the linear range of peak areas versus injection time (identical sample concentration) was found from 2 s to 40 s low pressure injection (0.5 p.s.i.; 1 p.s.i.= 6894.76 Pa). More than 40 s injection caused an overloading of the capillary and a break in this linearity. Good linear response of the areas versus the sample concentration was found under these conditions: with UV detection from 1 to 100 mg/l, 5 s injection, $r^2=0.997$ and with LIF detection (420 nm emission filter) from 0.5 to 10 mg/l, 10 s injection, $r^2=0.995$. The detection limit with maximal injection time (40 s) is thus 280 μg/l with UV detection (filter at 230 nm) and 37 μg/l with LIF detection.

MEKC was not used for quantification, but only to compare and confirm the presence of some photodegradation products. It gave a good linear response ($r^2>0.95$) of the areas versus the sample concentration in the concentrations range 1 to 5 mg/l with both detection methods, and the detection limit with 10 s injection time was calculated to be around 100 μg/l with LIF detection.

3.3. Photodegradation of enrofloxacin in presence of humic substances

Enrofloxacin showed rapid first-order kinetic photodegradation in distilled water with a half-life ($t_{1/2}$) of 2.0 h as measured with CZE. This value is very close to the estimated environmental half-life for enrofloxacin published in a previous study [7]. Only slight changes in photostability were observed in the presence of the low-molecular-mass fulvic acids ($t_{1/2}=1.9$ h). The presence of humic acid in the irradiated solution decreased the photodegradation kinetic significantly ($t_{1/2}=3.4$ h). Enrofloxacin strongly absorbs UV at a wavelength over 290 nm and is therefore very sensitive to a direct photodegradation pathway. The UV-absorbing humic acids are treating in competition with the biocide in absorbing

photons protecting the biocide from an effective photodegradation. This shielding effect is less pronounced in the case of fulvic acids because of their lower UV absorption. Photosensitizing by humic substances is not very important under these experimental conditions.

Fig. 5 shows the CZE and MEKC electropherograms of the irradiation solutions after 2 h. Differences can be observed in the degradation product patterns. Several photodegradation products including Nos. 8, 5 and ciprofloxacin can be clearly recognized without the addition of humic substances; the unknown degradation product ''compound A'' is present in highest concentration. In the presence of humic substances the degradation products disappear; only compound A and degradation product No. 5 are detectable. To explain these changes in terms of possible binding to the humic matrix, we investigated binding studies of the compounds to humic acids.

3.4. Binding of the fluoroquinolones to dissolved humic acids

Because of the high molecular mass and the polydisperse structure of humic acids, the complex-

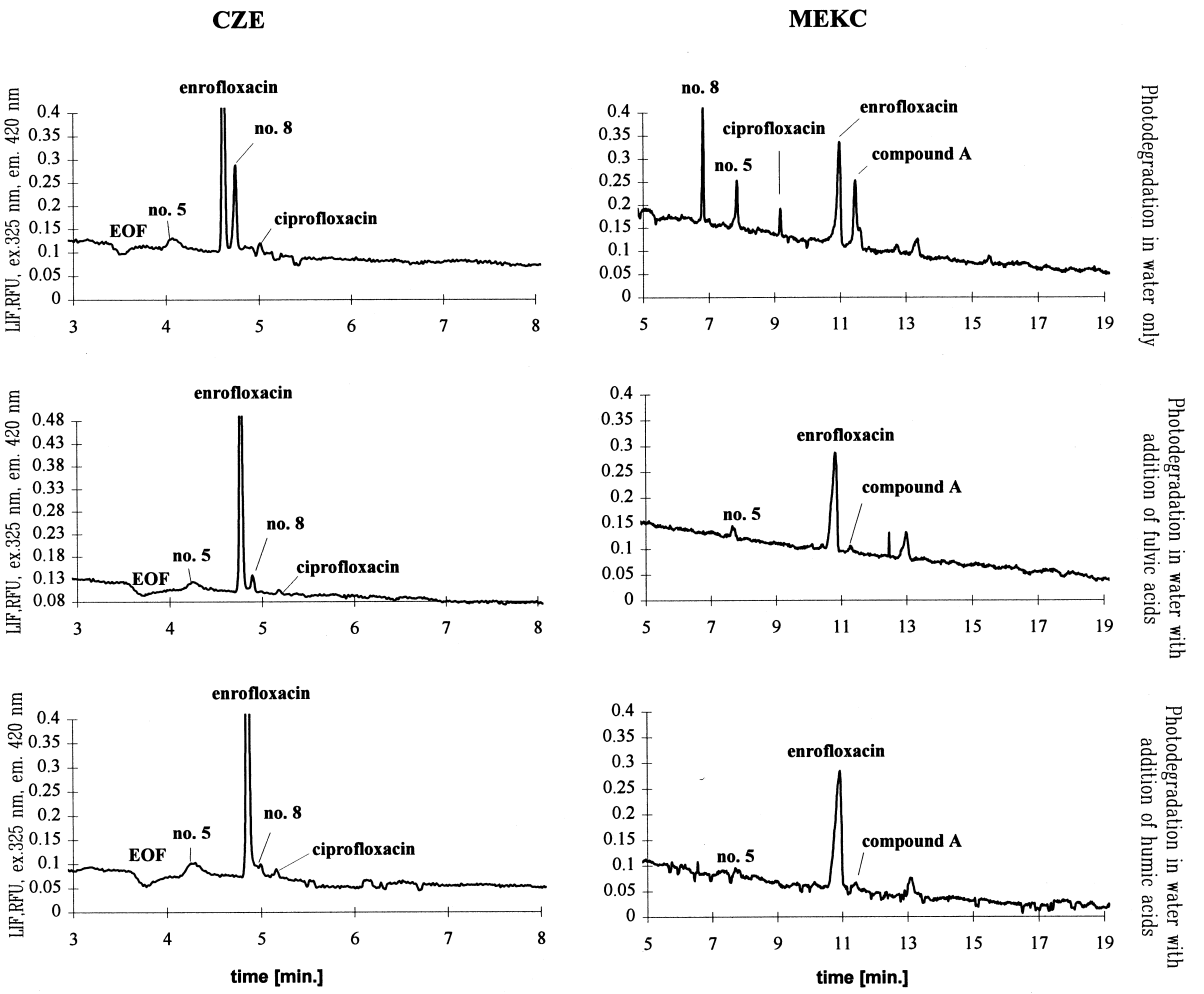


Fig. 5. MEKC and CZE separations of the 2-h irradiated enroflaxacin sample with and without humic substances (separation conditions as in Fig. 3).

ation does not occur at a 1:1 stoichiometry. The interactions of HS with biocides can be described by the partitioning of the substances between the aqueous and the organic humic phase. The partition coefficient K_p can thus be defined by the relation:

$$C_{ads} = K_p C_{eq} \quad (2)$$

where C_{ads} is the adsorbed biocide amount defined as $C_{ads} = ([P_{tot}] - [P])/L$ in g/kg, $[P_{tot}]$ is the initial biocide concentration in g/l, $[P]$ is the biocide concentration in solution at equilibrium in g/l, L is the ligand concentration of sorbent in kg/l; C_{eq} is the equilibrium concentration of the biocide in water defined as $C_{eq} = [P]$.

Under these conditions one finds:

$$K_p \frac{[P_{tot}] - [P]}{L[P]} \quad (3)$$

In affinity electrophoresis, the resulting effective electrophoretic mobility μ of the measured substance (directly proportional to the velocity) is a weighted product of the effective electrophoretic mobilities of all the free and bound forms of the analyte in the studied system

$$\mu = \frac{[P]}{[P_{tot}]} \cdot \mu_0 + \frac{[PL]}{[P_{tot}]} \cdot \mu_c \quad (4)$$

where μ_0 = electrophoretic mobility of the the free analyte for $[L] = 0$, μ_c is the electrophoretic mobility of the formed complex; $[PL]$ is the biocide concentration bound to the ligand with $[PL] = [P_{tot}] - [P]$)

Combination of Eqs. (3) and (4):

$$\mu = \frac{\mu_0 + \mu_c K_p [L]}{1 + K_p [L]} \quad (5)$$

assuming that the adsorption of the biocides is uniform over the humic fraction, the mobility of the formed complex becomes equal to the average mobility of the humic substances: $\mu_c = \mu_{HS} = -0.02032\ cm^{-2}/V\ s$ in carbonate buffer pH 9.2.

The changes in the effective mobility of enrofloxacin and its degradation products by addition of humic acid in the separation buffer (carbonate buffer pH 9.2) up to 1000 mg/l is shown as points in Fig. 6. The used buffer had a pH of 9.2 and the measurements were performed with one compound at the time. These experimental data points were fitted according to Eq. (5) and the fitted data are shown in Fig. 6 as lines; the corresponding calculated log K_p values are expressed in Table 2. Enrofloxacin and ofloxacin (essentially negatively charged) have the lowest affinity to the dissolved humic phase under these experimental conditions. The two decarboxylated compounds also show low affinity to the dissolved humic matrix, although the interaction of decarboxylated enrofloxacin (highest log K_{ow} and small partial positive charge) is slightly higher. The partition constants do not follow the same order as the log K_{ow} values (Table 1), so that hydrophobic effects are not dominant in these interactions. The binding strength may be correlated to the zwitterionic character of these molecules; compound Nos. 5 and 7 are present as zwitterions and have the highest dipolar character at pH 9.2 (Table 1). The binding strength can be correlated to the partial positive charge of the analytes (charge repartition in Table 1). The steric effects shown for the interactions of compound Nos. 5 and 7 with the SDS micelles do not exist with humics. The correlation (linear) between the log K_p and the partial positive charge of the analytes is only of $r^2 = 0.63$, so that the interactions are probably due to a combination of electrostatic interactions between the positively charged amine group and negatively charged head groups in the humic structures (OH, COOH) and other kind of active or passive adsorption types like hydrogen bonding, charge transfer or hydrophobic effect (inclusions in hydrophobic HS sites). The mechanisms might be comparable with the one occurring in anionic micelles. On the one hand, charge repulsions and hydrophobic aggregations govern the orientation of the analytes towards the

Table 2
Calculated partition constants for the quinolones to the dissolved humic acid (pH 9.2)

Compound	Log K_p (±0.1)
Enrofloxacin	−3.95
Ciprofloxacin	−3.60
Norfloxacin	−3.30
Danofloxacin	−3.20
Ofloxacin	−4.00
No. 5	−2.70
No. 7	−3.20
No. 8	−3.75
Decarboxylated No. 8	−3.90
Decarboxylated enrofloxacin	−3.60

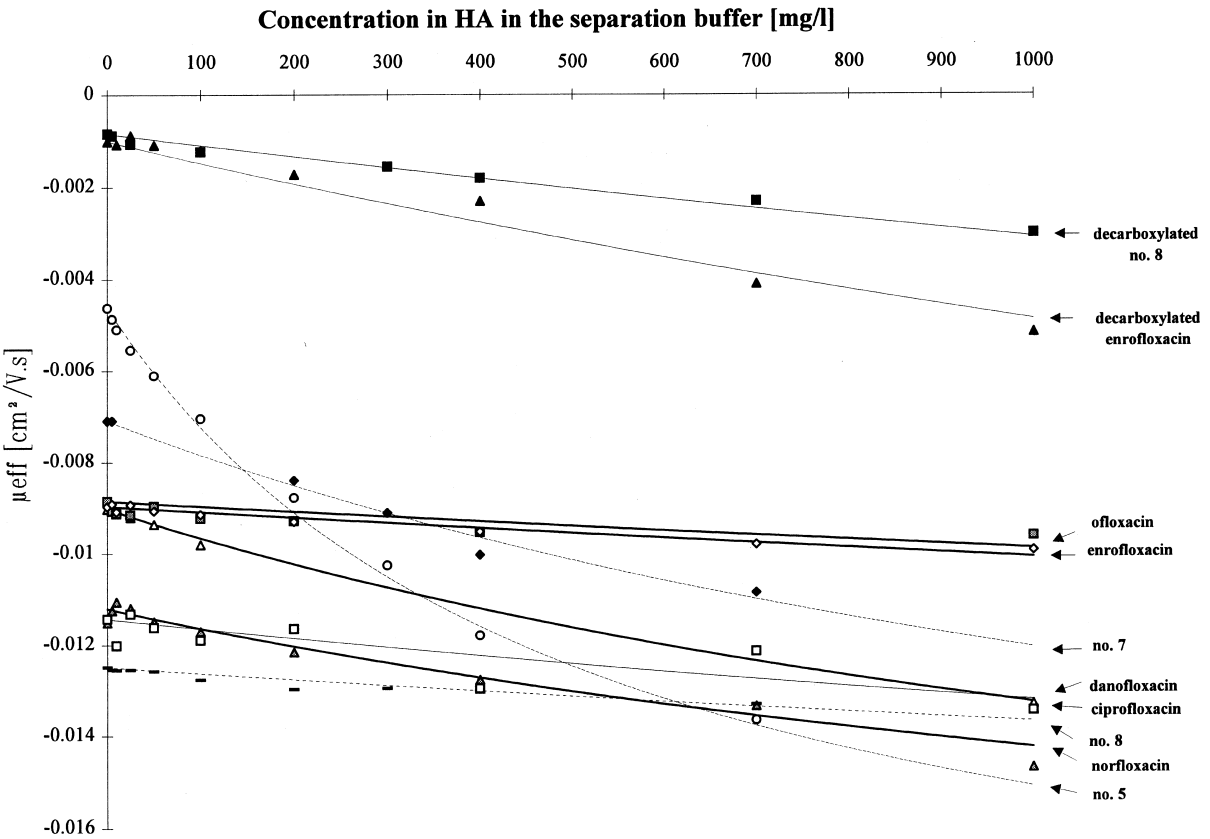


Fig. 6. Changes in effective mobility of enrofloxacin and its degradation products by addition of humic acid in the separation buffer (carbonate buffer pH 9.2) (LIF detection ex. 325 nm, em. 420 nm; 20 kV, 30°C, 25 m*M* carbonate buffer pH 9.2, sample concentration 5 mg/l).

reactive sites of the humics (negatively charged humic sites, hydrophobic sites), on the other hand electrostatic and hydrogen bonding may attract and hold the analyte molecules close to humic macromolecules. Lower charge repulsion effects could be expected at neutral pH (pH of the irradiated solutions), because of the lower ionization degree of both the fluoroquinolones and the humic substances, implicating stronger binding effects (more hydrophobic sites on the humics).

Compound 5 is the only photodegradation product that can still be detected in higher concentrations with the presence of humic substances. A possible explanation is that the higher binding of compound 5 to the humic phase protects it more from photodegradation than the other degradation products.

4. Conclusions

Capillary electrophoresis methods for the separation and determination of fluoroquinolones were developed in CZE and MEKC mode. A good estimation of the mobility of these biocides was achieved from charge density parameters ($Z/M^{2/3}$, Z/V_m and Z/S_m), and this technique can be an effective tool when developing CE methods for new compounds (i.e., degradation products of compounds of interest).

The photodegradation of enrofloxacin was investigated under environmental solar conditions. A decrease in the photodegradation rate was observed in the presence of humic acids because of shielding effects leading to lower direct photolysis of enrofloxacin. Binding studies between all compounds and

humic acids were conducted with capillary electrophoresis and indicated rather an electrostatic type of interactions. The stronger binding of some degradation products seems to allow a relative protection from their photodegradation. Photodegradation plays an important role as a degradation pathway for quinolones in the environment.

Acknowledgements

Thanks are due to Heidi Neumeir and Alexander Krainz of the Institute for Ecological Chemistry–GSF for skillful technical assistance and for providing the atmospheric data respectively. We acknowledge Bayer, Leverkusen for providing enrofloxacin, ciprofloxacin and reference compounds.

References

[1] D.C. Hooper, J.S. Wolfson, Quinolones Antimicrobial Agents, American Society for Microbiology, Washington, 1993.
[2] M. Scheer, Vet. Med. Rev. 2 (1987) 104.
[3] S.A. Brown, J. Vet. Pharmacol. Ther. 19 (1996) 1–14.
[4] J. Espinasse, Vet. Microbiol. 35 (1993) 289–301.
[5] W. Gau, H.J. Ploschke, K. Schmidt, B. Weber, J. Liq. Chromatogr. 8 (1985) 485–497.
[6] A. Nowara, J. Burhenne, M. Spiteller, J. Agric. Food Chem. 45 (1997) 1459–1463.
[7] J. Burhenne, M. Ludwig, P. Nikoloudis, M. Spiteller, ESPR-Environ. Sci. Pollut. Res. 4 (1997) 10–15.
[8] J. Burhenne, M. Ludwig, M. Spiteller, ESPR-Environ. Sci. Pollut. Res. 4 (1997) 61–67.
[9] K. Torniainen, J. Mattinen, C.P. Askolin, S. Tammilehto, J. Pharm. Biomed. Anal. 15 (1997) 887–894.
[10] Y. Yoshida, E. Sato, R. Moroi, Arzneim.-Forsch./Drug Res. 43 (1993) 601–606.
[11] J. Burhenne, M. Ludwig, M. Spiteller, presented at Umwelt und Chemie '96, Umwelttagung der GDCh, Ulm, 7–10 October 1996, poster.
[12] K.-H. Bannefeld, H. Stass, G. Blaschke, J. Chromatogr. B 692 (1997) 453–459.
[13] Ph. Schmitt, A.W. Garrison, D. Freitag, A. Kettrup, J. Chromatogr. A 723 (1996) 169–177.
[14] Ph. Schmitt, T. Poiger, R. Simon, A.W. Garrison, D. Freitag, A. Kettrup, Anal. Chem. 69 (1997) 2559–2566.
[15] A.W. Garrison, Ph. Schmitt, D. Martens, A. Kettrup, Env. Sci. Technol. 30 (1996) 2449–2455.
[16] Ph. Schmitt, Y. Sanlaville, J. Lintelman, D. Freitag, A. Kettrup, J. Chromatogr. A 709 (1995) 215–225.
[17] Z. El Rassi, Electrophoresis 18 (1997) 2465–2481.
[18] G. Rippel, H. Cortjens, H.A.H. Billiet, J. Frank, Electrophoresis 18 (1997) 2175–2183.
[19] Ph. Schmitt, I. Trapp, A.W. Garrison, D. Freitag, A. Kettrup, Chemosphere 35 (1997) 55–75.
[20] Ph. Schmitt, A.W. Garrison, D. Freitag, A. Kettrup, Fresenius J. Anal. Chem. 354 (1996) 915–920.
[21] S. Fu, C.A. Lucy, Anal. Chem. 70 (1998) 173–181.
[22] R.E. Offord, Nature 211 (1966) 591.
[23] B.J. Compton, J. Chromatogr. 559 (1991) 357.
[24] J. Fekete, G. Morovjan, F. Csizmadia, F. Darvas, J. Chromatogr. A 600 (1994) 33–46.
[25] K. Takacs-Novak, B. Noszal, I. Hermecz, G. Kereszturi, B. Podanyi, G. Szasz, J. Pharm. Sci. 79 (1990) 1023–1028.
[26] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 113–116.
[27] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834–841.
[28] H. Süsse, H. Müller, Fresenius J. Anal. Chem. 352 (1995) 470–473.
[29] Ph. Schmitt, A.W. Garrison, D. Freitag, A. Kettrup, J. Chromatogr. A 792 (1997) 419–429.
[30] C. Desiderio, S. Fanali, Electrophoresis 13 (1992) 698–700.
[31] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis – Principles and Practice, Springer, Berlin, 1993.
[32] C. Tanford, The Hydrophobic Effect – Formation of Micelles and Biological Membranes, Wiley-Interscience, New York, 1980.
[33] M.F. Vitha, J.D. Weckwerth, K. Odland, V. Dema, P.W. Carr, J. Phys. Chem. 100 (1996) 18823–18828.