Determination of Ofloxacin in Pharmaceuticals, Human Urine and Serum Using a Potentiometric Sensor

A. M. Pimenta,*[a] *M. R. S. Souto*,[b] *R. I. L. Catarino*,[a] *M. F. C. Leal*,[a] *J. L. F. C. Lima*[c]

[a] REQUIMTE, CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal
[b] CBQF, CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal
[c] REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, 4090-030 Porto, Portugal
*e-mail: apimenta@ufp.edu.pt

Received: September 30, 2010
Accepted: December 1, 2010

Abstract
An ofloxacin-selective electrode was developed for application in pharmaceutical preparations, serum and urine analysis. The electrode is based on tetrakis[3,5-bis(trifluoromethyl)phenyl]borate as ion exchanger in a poly(vinyl chloride) membrane. The sensor displayed a Nernstian response for ofloxacin over a wide concentration range (3×10^{-6}–1×10^{-2} mol L^{-1}) with a slope of 55 ± 1 mV per decade of concentration and a practical detection limit of 1×10^{-6} mol L^{-1}. The electrode was successfully applied to the determination of ofloxacin in samples with no pretreatment steps, adequate accuracy and precision and recoveries within the intervals of 94–107 %.

Keywords: Ofloxacin, Ion-selective electrode, Potentiometry, Pharmaceuticals, Serum, Urine, PVC membrane

DOI: 10.1002/elan.201000608

1 Introduction

Ofloxacin (OFLX) [(±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxacine-6-carboxylic acid] (Figure 1), is a synthetic quinolone antimicrobial agent.

It exhibits broad-spectrum activity against both Gram-positive and Gram-negative organisms, as well as atypical pathogens such as *Mycoplasma, Chlamydia* and *Legionella* [1]. The bactericidal action of OFLX results from its interference with enzyme DNA gyrase and topoisomerase IV, needed for the synthesis of bacterial DNA. OFLX is used in both human and veterinary medicine for the treatment of a wide variety of diseases, namely urinary, respiratory and gastrointestinal tract infections [2–5]. It has two optical isomers: levofloxacin (LVFX), the (−)-(*S*)-enantiomer (which is the more active isomer), and dextrofloxacin, the (+)-(*R*)-OFLX. Currently, the drug is marketed by a variety of brand names as well as generic drug equivalents as a racemic mixture (consisting of equal amounts of the enantiomers) or only as *S*-isomer. According to literature, the (−)-(*S*)-enantiomer of the drug, is approximately two-fold more potent than the racemic mixture [6–8].

OFLX is rapidly and almost completely absorbed. Following oral administration the bioavailability is approximately 98 % and peak plasma concentrations are usually attained one to two hours after an oral dose. This substance has a pyridobenzoxazine ring that appears to decrease the parent metabolism. The drug undergoes limited metabolism and is primarily excreted as unchanged in urine, between 65 % and 80 % of an administered oral dose of OFLX is excreted via the kidneys within 48 hours of dosing, thus producing high levels of active drug in urine with excellent cure rates in the treatment of urinary tract infections [2,9]. Pharmacokinetics studies point out that less than 5 % of the administered dose is recovered in urine as the desmethyl or N-oxide metabolites, and such low metabolite concentration are of no clinical importance [9,10].

The actual therapeutic importance of this drug requires selective, rapid and accessible analytical methods for its determination in pharmaceutical formulations and in biological matrices. A review of the literature revealed that most analytical methods resort to the use of high performance liquid chromatography (HPLC) [11–14] and capilary electrophoresis (CE) [15–20] for this purpose. However, in spite of their high selectivity and sensitivities, these techniques are time-consuming, require complex sample procedures and involve the use of large amounts of reagents and organic solvents thus incurring in considerable costs. Other alternatives described include spectrophotometric [21–23], fluorimetric [24–26], chemiluminesce [27–29], voltammetric [30–32], and immunoassay [33] methodologies. Most of them present several disadvantages such as use of expensive equipment, inadequate

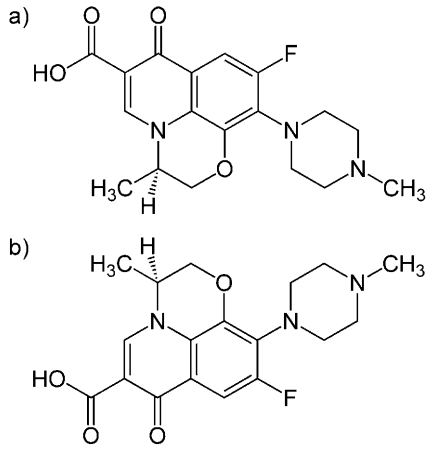

Fig. 1. Chemical structure of ofloxacin enantiomers. (a) *R*-enantiomer; (b) *S*-enantiomer or levofloxacin.

selectivity, requirement of long sample pretreatment or even complex treatment of the analytical results.

In recent decades ion-selective electrode (ISEs) have been widely used in several fields of modern analytical chemistry, including pharmaceutical [34] and biological analysis [35,36], thanks to their simple design, low cost, relatively short response times, limited or no sample preparation steps, adequate selectivity, good accuracy, wide linear concentration range and possible interfacing with automated and/or computerised systems [37,38]. Synthetic receptors (ionophores) that selectively bind to the target analytes have been reported for over 60 species [39,40].

The aim of this work was to develop a new simple potentiometric PVC-membrane sensor for the simple, sensitive, rapid and selective determination of OFLX in pharmaceuticals and in more complex samples like biological fluids. As the ionophore nature has a critical role in the ISEs performance, different possible ionophores, functionalized cyclodextrins (CDs), vancomycin (VAC) and potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (KTFPB), were tested. The selected membrane consisted of liquid-plasticizer PVC and was based on lipophilic tetrakis[3,5-bis(trifluoromethyl)phenyl] borate as ion exchanger.

2 Experimental

2.1 Reagents and Solutions

Deionised water (with a specific conductance lower than 0.1 μS/cm) and analytical grade chemicals without additional purification were used. For ISEs membranes preparation the following reagents were used: high molecular weight poly(vinyl chloride) (PVC, Fluka 81392) or carboxilated high molecular weight poly(vinyl chloride) (PVC-COOH, Jansen 1833195) as immobilising matrix; potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (Fluka 60588), tetradodecylammonium bromide (TDB, Fluka 87249), methyl- β-cyclodextrin (methyl- β-CD, Aldrich 332615), (2-Hydroxypropyl)-β-cyclodextrin (HP-β-CD, Fluka 56332), carboxymethyl-β-cyclodextrin (COOH-β-CD, Fluka 21906) or vancomycin hydrochloride (Fluka 94747) as additives or ionophores; and, 2-nitrophenyloctyl ether (ONPOE, Fluka-73732) or bis(2-ethylhexyl) sebacate (DOS, Fluka 84818) as mediator solvent. The solid polymers were dissolved in tetrahydrofuran (THF, Sigma-Aldrich 34865).

To enable simultaneous pH and ionic strength adjustments, a 0.1 mol L^{-1} NaH_2PO_4 solution was prepared and the pH adjusted to 2.5 with concentrated H_3PO_4 solution. Standard 1×10^{-2} mol L^{-1} OFLX (Sigma O8757) or LVFX (Fluka 28266) solutions were prepared daily by weighing of the solid and dilution in the buffer solution.

All inorganic interferent standard solutions were prepared of the analytical grade chloride salts.

The pharmaceutical preparations used were as follows: Tarivid (containing 200 mg OFLX/tablet), Bioquil (400 mg OFLX/tablet), Ofloxacina Merck (200 mg OFLX/tablet), Tavanic (500 mg LVFX/tablet), Loxadin (500 mg LVFX/tablet) and Levofloxacina J Neves (containing 500 mg LVFX/tablet).

Human urine samples were collected from 10 healthy volunteers. For analytical measurements, a pool of these fluids was used.

Synthetic serum samples were prepared as follows: 138 mmol L^{-1} NaCl; 2.8 mmol L^{-1} KCl, 1.3 mmol L^{-1} KH_2PO_4; 2.5 mmol L^{-1} $CaCl_2{\cdot}2H_2O$; 1.0 mmol L^{-1} $MgSO_4{\cdot}7H_2O$ and 6 % bovine serum albumine [41].

Urine and serum samples were spiked with appropriate amounts of analyte, to match biological concentrations usually found in these biological fluids.

2.2 Membrane and Sensor Preparation

Polymeric membranes were prepared by dissolution of the ionophore and the additive in the mediator solvent. Powdered PVC (0.16 g) and 6 mL of tetrahydrofuran were subsequently added and thoroughly mixed to obtain a homogeneous solution. Membrane composition of the several types of OFLX-selective electrodes tested is presented in Table 1.

The membrane solutions were then applied dropwise on the conductor support, made up of a mixture of epoxi resin (Araldite) with graphite powder constructed following the methodology previously described [42]. After tetrahydrofuran evaporation, successive applications of the sensor solutions were performed until a membrane 1 mm thick was obtained. Next, they were allowed to dry (at room temperature) overnight until total evaporation of tetrahydrofuran occurred. Before use, the electrodes were soaked in a 1×10^{-3} mol L^{-1} OFLX solution and conditioned until a potential drift lower than 0.1 mV min^{-1} was achieved (approximately 30 minutes).

Table 1. Membrane composition (% w/w) of the several types of ion-selective electrodes.

Electrode type	Ionophore (1–2%)	Additive (0.4%)	Mediator solvent (66%)	Immobilising matrix (33%)
A	VAC		ONPOE	PVC
B	methyl-β-CD		ONPOE	PVC
C	HP-β-CD		ONPOE	PVC
D	COOH-β-CD		ONPOE	PVC
E	VAC		DOS	PVC
F	methyl-β-CD		DOS	PVC
G	HP-β-CD		DOS	PVC
H	COOH-β-CD		DOS	PVC
I	VAC	TDB	ONPOE	PVC
J	methyl-β-CD	TDB	ONPOE	PVC
K	HP-β-CD	TDB	ONPOE	PVC
L	COOH-β-CD	TDB	ONPOE	PVC
M	VAC	KTFPB	ONPOE	PVC
N	methyl-β-CD	KTFPB	ONPOE	PVC
O	HP-β-CD	KTFPB	ONPOE	PVC
P	COOH-β-CD	KTFPB	ONPOE	PVC
Q		KTFPB	ONPOE	PVC
R	VAC	KTFPB	ONPOE	PVC-COOH
S	methyl-β-CD	KTFPB	ONPOE	PVC-COOH
T	HP-β-CD	KTFPB	ONPOE	PVC-COOH
U	COOH-β-CD	KTFPB	ONPOE	PVC-COOH
V		KTFPB	ONPOE	PVC-COOH
W			ONPOE	PVC
X			ONPOE	PVC-COOH

2.3 Sample Preparation

2.3.1 Procedure for the Analysis of Ofloxacin in Pharmaceutical Preparations

Ten tablets of each pharmaceutical were weighed, ground and mixed in a porcelain mortar. An appropriate amount of the resulting powder was weighed (equivalent to 150 mg OFLX/LVFX), dissolved in buffer solution and then transferred to 50 mL volumetric flasks. Finally, different accurate volumes of these mixtures were diluted in the same buffer, to prepare solutions with a final expected OFLX/LVFX concentration from 5×10^{-5} to 3×10^{-4} mol L^{-1}. These drug levels are included in the working range of the electrodes and therefore potentiometric measurements were carried out in the linear nernstian zone of the analytical curve. Recovery studies were also performed on each of the pharmaceuticals solutions prepared above. An aliquot of 25.0 mL of each pharmaceutical sample solution, was spiked with different volumes of OFLX 1×10^{-2} mol L^{-1} standard (0.30–3.0 mL) and analysed in triplicate.

2.3.2 Procedures for Analysis of Ofloxacin in Urine and Serum

Human urine and serum samples were spiked with adequate amounts of OFLX 1×10^{-2} mol L^{-1} standard solution to meet active therapeutical concentrations [10,11]. Final added OFLX concentrations in urine and serum samples were 7×10^{-4} to 2×10^{-3} and 8×10^{-6} to 2×10^{-5} mol L^{-1}, respectively. Aside from a dilution step with buffer solution, 1:25 or 1:10 for urine matrices and 1:2 for serum samples, no other pretreatment procedure was required. The potential of different solutions was measured using the general procedure described and the OFLX analytical concentration obtained either by direct interpolation in the analytical curve or by the standard addition method (for OFLX level $<4\times10^{-5}$ mol L^{-1} in urine samples and $<4\times10^{-6}$ mol L^{-1} in serum samples). In this latter situation, volumes of 25.0 mL of each urine diluted sample were supplemented with increasing volumes of a 1×10^{-2} mol L^{-1} standard OFLX solution (0.10; 0.30. 0.80 and 1.5 mL) and the new potential measured under the same conditions. Each sample was analysed in quadruplicate and the amount of OFLX was calculated by extrapolation from all the data points obtained. Regarding serum samples, a similar procedure was used. The only differences were that a 20.0 mL volume of diluted sample was used and the additions of the 1×10^{-2} mol L^{-1} standard OFLX solution were 10, 20, 30 and 40 μL for OFLX serum concentrations lower than 4×10^{-6} mol L^{-1} and 100, 200, 300 and 400 μL for the other serum samples.

2.4 Apparatus and Measurements

A Crison micro-pH, model 2002 voltmeter (±0.1 mV sensitivity), was used to measure the potential differences between an Orion (model 900200) double-junction Ag/AgCl reference electrode and the proposed indicator electrodes. The outer compartment of the reference electrode contained the buffer solution. pH measurements

were performed with a WTW model SenTix 41 glass electrode.

To establish general analytical performance characteristics of the sensor systems, OFLX-selective and reference electrodes were immersed in phosphate buffer solution and adequate volumes of a standard OFLX solution were added, while stirring, to cover the concentration range from 10^{-7} to 10^{-2} mol L^{-1}.

Calibration plots with OFLX concentrations between 1×10^{-5} and 2×10^{-3} M were performed for sample analysis.

3 Results and Discussion

3.1 Development and Optimization of Sensor Membranes

In preliminary studies, different possible ionophores, some cyclodextrins and vancomycin, were tested.

Cyclodextrins are a family of cyclical oligosaccharides, with torus-like form, that allows the inclusion of several organic compounds of adequate size and polarity in a "host–guest" type of structure. This property makes these receptors in an interesting class of species to be studied as electrochemical sensors for wide range of alkyl and arylammonium ions [43,44]. Additionally, some authors reported the use of CDs and VAC as chiral selectors in chromatographic systems for HPLC and CE enantioselective determination of OFLX [17,18,20,45]. Taking these into account, the first trial was to develop selective electrodes to OFLX using three functionalized lipophilic CDs (methyl-β-CD, HP-β-CD and COOH-β-CD) and VAC as sensor ionophores. Two different plasticizers (with very different dielectric constants) as mediator solvents (ONPOE and DOS) were also tested. PVC was used as a support matrix (Table 1). None of the membrane formulations tested (electrodes A-H) showed significant activity for OFLX and thus were no further used.

Several authors reported that the addition of a low percentage of lipophilic species as an additive can contribute to a decrease of the membrane resistance and a concomitant improvement in the slope and selectivity of the electrodes [46]. The inclusion of a small amount of KTFPB or TDB, as fixed ionic site additives, was then tested. No reaction to OFLX was shown by the electrodes with TDB (types I–L) but, surprisingly, regardless of the sensor system used, all the electrodes containing KTFPB (types M–P) presented similar analytical performance characteristics (with near Nernstian slopes, comparable practical detection limits and analogous pH operational range) (Table 2).

In an attempt to understand the role of the KTFPB in the recognition mechanism of the membrane, new electrodes contained only this anionic exchanger were assembled. This series of electrodes (type Q) displayed analytical features similar to the ones previously described (types M–P) (Table 2), suggesting that the effect of CDs or VAC on potentiometric activity might be null and that the performance characteristics of all these sensors could be due entirely to the use of the additive.

Ofloxacin is an organic amine positively charged under a relatively wide pH range. It can react with the anionic tetraphenylborate group present in the additive to form lipophilic water-insoluble ion-pair complexes [47]. The potentiometric activity of electrodes with KTFPB could therefore be attributed to an ion exchange mechanism. This ion-pair would be obtained in situ, after soaking the PVC membranes containing KTFPB in 1×10^{-3} mol L^{-1} OFLX solution for electrode conditioning prior to its use.

As the inclusion of either CDs or VAC in the membranes did not improve the general analytical characteristics of the electrochemical devices, KTFPB electrodes (type Q) were selected to carry on the study.

The effect of the immobilising matrix on potentiometric response of the KTFPB electrodes was then assessed. Some authors report improvements in electroanalytical characteristics when using carboxylated high molecular weight poly(vinyl chloride) [48]. In this case, the input in negative charge (due to the carboxylic group), could also benefit the response mechanism. No difference in the membranes response was obtained with formulations using PVC-COOH (types R–V) instead of PVC.

The response of blank membranes, containing only solvent ONPOE and either PVC (type W) or PVC-COOH (type X), was also studied. Previous studies point out that ionic impurities present in either the mediator solvent or PVC can generate permeselectivity in the membranes towards cationic and anionic species [49,50]. No answer was obtained with blank PVC electrodes. Blank PVC-COOH membranes exhibited a weak response to OFLX (slope of 46 ± 1 mV/decade of concentration) that decreased rapidly throughout the following days (less than half of the initial slope was obtained in 2 to 3 days).

Membranes with PVC-COOH, similar ionophore (CDs or VAC) content and no anionic additive were also constructed (data not shown). Their potentiometric response was similar to the one exhibited by blank PVC-COOH electrodes (type X) supporting the idea that both CDs and VAC had no potentiometric activity.

Finally, to assess the effect of the plasticizer solvent in these functioning PVC-COOH membranes, ionophores were tested with DOS as mediator solvent. No analytical response was observed, probably due to the inadequate physicochemical properties of DOS, namely its low dielectric constant.

After this study, type Q electrodes were chosen as having the most suitable composition to carry out the rest of the work.

3.2 General Working Characteristics

General working characteristics were determined performing regular calibrations with OFLX solutions (concentration range of 1×10^{-7} to 1×10^{-2} mol L^{-1}) in solutions with adjusted pH and ionic strength, according to IUPAC recommendations [51]. Data obtained, from the

Table 2. General working characteristics of the OFLX-sensors. Data obtained with at least three units of each type of electrodes; pH and ionic strength adjusted with H_3PO_4/NaH_2PO_4 buffer solution ($I=0.1$ mol L^{-1} and pH 2.5). *PDL*: practical detection limit; *LLLR*: lower limit of linear response.

Parameter	Electrode type				
	M	N	O	P	Q
Slope (mV decade^{-1}) [a]	55 ± 1	54.4 ± 0.4	54 ± 2	54.2 ± 0.9	54 ± 1
Linear correlation coefficient (R^2) [a]	0.9997 ± 0.0002	0.9999 ± 0.0001	0.9999 ± 0.0001	0.9997 ± 0.001	0.9998 ± 0.0001
PDL (mol L^{-1})	3×10^{-6}	7×10^{-6}	7×10^{-6}	8×10^{-6}	1×10^{-6}
LLLR (mol L^{-1})	1×10^{-5}	3×10^{-5}	1×10^{-5}	2×10^{-5}	3×10^{-6}
Reproducibility (mV day^{-1})	1.4	0.9	0.9	1.2	0.7
Working pH range [b]	1.3–5.2	1.5–5.8	1.5–5.2	1.5–5.2	1.1–5.6
Response time (s)	<10	<10	<10	<10	<10
Lifetime (months)	6	6	6	6	6

[a] Average value $\pm$ standard deviations of 20 determinations over a period of 6 months. [b] Results corresponding to 5×10^{-3} and 5×10^{-4} mol L^{-1} and of OFLX solutions.

evaluation of at least three units of each type are presented in Table 2. No significant differences between electrodes with diverse membrane composition were noticed regarding general calibration parameters (namely slope, lower limit of linear response and practical detection limit). This could be ascribed to the fact that these set of sensors had similar KTFPB amounts (0.4 %). Electrodes possessing different KTFPB concentrations were also assembled −0.04, 0.2 and 1 %. As expected, the only difference between these units regarded their lifespan: 5, 6 and more than 8 months, respectively. This fact could be attributed to the higher amount of ion exchanger in its composition. Electrodes displayed a wide range of linear response (3×10^{-6} to 1×10^{-2} mol L^{-1}), with a near Nernstian slope of 55 ± 1 mV decade^{-1} and a practical detection limit of 1×10^{-6} mol L^{-1}. It should also be mentioned that these sensors had a short response time (less than 10 s) and exhibited a day-to-day reproducibility of about ±0.7 mV.

3.3 Influence of pH

The effect of pH in the electrode response was examined over the pH range of 1–13. Experiments were conducted with two electrodes of each type, for two concentration levels of the primary ion: 5×10^{-4} and 5×10^{-3} mol L^{-1}. The pH of the solution was adjusted adding small volumes of concentrated sulphuric acid or sodium hydroxide solutions to the aqueous OFLX solution. The potential changes as a function of pH were recorded. The operational pH range was established when the change in potential did not exceed more than 5 mV. Results of a typical profile for 5×10^{-4} and 5×10^{-3} mol L^{-1} OFLX solutions, using electrode type Q are shown in Figure 2.

As seen, the potential remains almost constant over the pH range of 1.1 to 5.6 which can be taken as working pH range of the electrode. OFLX has zwitterionic properties by virtue of carboxyl group being deprotonated at basic pH values ($pKa_1=6.0$) and its amino function in position 4 of the piperazine ring being positively charged under acidic conditions ($pKa_2=8.0$). So, at the pH interval mentioned above, the OFLX molecules are protonated and positively charged and can be sensed by anion-exchanger KTFPB present in the electrodes. For pH values higher than 6 a severe decrease of the cationic form of OFLX occurs since the analyte molecules are mainly at the zwitterionic form. Having no net charge, OFLX has no ability to form the ion-pair complex and therefore is no longer detected, that is, a marked decrease in the electrode response is observed. Considering this behaviour a pH 2.5 phosphate buffer solution was used throughout the rest of the work. This solution acted both as an ionic strength and pH adjuster. The same pattern of pH dependence was found for other electrodes types (electrodes M–V) (Table 2).

3.4 Interference Studies

Potentiometric selectivity coefficients (log $K_{OFLX,I}^{pot}$) against several species were evaluated using the separate solutions method (SSM) at two concentrations levels (1×10^{-4} and 1×10^{-3} mol L^{-1}) of primary (OFLX) and interfering ion (I), according to IUPAC guidelines [52]. Table 3 presents results obtained for main inorganic cations present in biological fluids (Group I and II cations and ammonium ion) and two organic cations (phenylethylamine and epinephrine) having a chemical structure similar to that of the target species tested.

Data obtained point out that all systems with KTFPB showed a similar pattern of interference with a small response to the interfering species (selectivity coefficients obtained in the order of 10^{-2} or smaller). This confirmed the fact that the active substance is actually the ion exchanger KTFPB and reinforces the choice of type Q membranes over those that also included CDs or VAC (electrode types M–O). Additionally, the substitution of the immobilizing matrix (type V) did not result in an improvement in selectivity (Table 3).

As blank PVC-COOH membranes initially exhibited some activity towards OFLX, their selectivity coefficients were also determined. As expected, they were much larger than those obtained for the membranes with KTFPB, highlighting their unsuitability for analytical purposes.

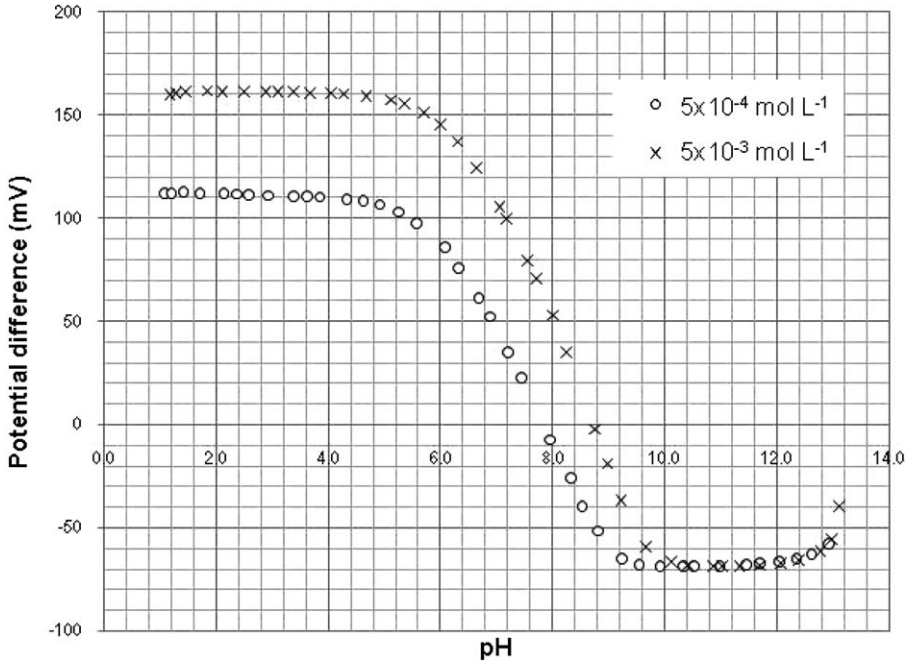

Fig. 2. Effect of pH on the response of electrodes type Q in 5×10^{-4} and 5×10^{-3} mol L^{-1} OFLX solutions.

The selectivity pattern mentioned for the KTFPB units follows the sequence: $Mg^{2+} < Ca^{2+} < Li^{+} < Na^{+} < NH_4^{+} < K^{+} <$ phenylethylamine < epinephrine. This order is related to the mechanism of action of the ion exchanger, which depends on physico-chemical characteristics of the ion exchange process at the membrane-sample solution interface, the mobilities of the respective ions in the membrane and the hydrophobic interactions between the primary ion and the organic membrane. The high selectivity for inorganic ions was attributed to the differences in ionic size, and consequently their mobilities and permeability, as compared to those of OFLX cation. Regarding organic molecules, the lower selectivity is mainly due to the similarity in polarity and to the moderately hydrophobic nature of these molecules relative to OFLX cation.

As OFLX is a racemic mixture, consisting of equal amounts *R*- and *S*-enantiomers, the log $K_{S\text{-isomer},R\text{-isomer}}^{pot}$ was also assessed as a mean of determining the enantioselectivity of the assembled membranes. The *S*-enantiomer was considered the primary species and the *R*-enantiomer the interferent ion (Table 3). As the mechanism of action of these membranes is based on electrostatic interactions, no chiral selectivity was observed and the electrodes response was the same for both isomers (log $K_{S\text{-isomer},R\text{-isomer}}^{pot} \approx 0$).

Considering data obtained for all the species tested, the proposed KTFPB membrane electrode (type Q) presents an adequate selectivity toward OFLX.

The effect of common excipients and adjuvants used in ofloxacin pharmaceutical formulations, namely, silicon dioxide, glucose, lactose, starch, magnesium stearate, polyethylene glycol and titanium dioxide was also studied. Samples contained a fixed amount of OFLX (1×10^{-4} mol L^{-1}) and variable excipients concentrations were measured. The tolerated limited for excipient was taken as the largest amount yielding a relative error lower than 5%. None of the aforementioned species cause interference up to excipient/ofloxacin ratio (w/w) of 100, which is a much higher value than that found in commercial formulations.

Ofloxacin has the potential to form stable coordination compound with Fe^{3+}, Al^{3+}, Cu^{2+} and Ni^{2+}. Solutions with a constant level of OFLX (1×10^{-4} mol L^{-1}) and increasing amounts of metals were prepared. Nickel(II) and aluminum(III) do not interfere up to a molar metal/OFLX ratio of 100 fold. Copper(II) and aluminum(III) start to interfere negatively at a molar ratio higher than 10 fold. Both ratios are higher than those found in all analysed samples. So no interference from these cations was expected.

Ofloxacin degradation products (decarboxy ofloxacin, 9-piperazino ofloxacin, des-methyl ofloxacin, and ofloxacin-*N*-oxide) may be present in pharmaceuticals and biological samples. However, as pointed out previously, OFLX undergoes limited metabolism (less than 5% of the administered dose is recovered in urine as metabolites) and degradation and only trace levels of these species are found in both matrices [9–11]. At such low concentrations the extent of their influence in OFLX quantification is so limited that it can be considered to have no significant effect on the analytical signal.

4 Analytical Applications

The present KTFPB membrane electrode was successfully applied to the determination of OFLX in pharmaceutical preparations and different biological fluids (human

Table 3. Potentiometric selectivity coefficients ($\log K_{OFLX,I}^{pot}$) of the OFLX electrodes (mean ± standard deviation of 4 results obtained with two units).

	Electrode type											
	M		N		O		P		Q		V	
Inteferent ion (I)	1×10^{-4} M	1×10^{-3} M	1×10^{-4} M	1×10^{-3} M	1×10^{-4} M	1×10^{-3} M	1×10^{-4} M	1×10^{-3} M	1×10^{-4} M	1×10^{-3} M	1×10^{-4} M	1×10^{-3} M
Mg^{2+} [a]	−4.91 ± 0.42	−4.97 ± 0.85	−5.24 ± 0.23	−5.55 ± 0.11	−5.26 ± 0.09	−5.81 ± 0.20	−5.30 ± 0.08	−5.87 ± 0.16	−4.37 ± 0.12	−5.34 ± 0.13	−3.99 ± 0.58	−3.83 ± 0.83
Ca^{2+} [a]	−3.12 ± 0.38	−4.11 ± 0.70	−4.45 ± 0.10	−4.98 ± 0.12	−3.69 ± 0.09	−4.79 ± 0.13	−4.37 ± 0.16	−5.11 ± 0.17	−4.84 ± 0.24	−5.50 ± 0.17	−3.43 ± 0.38	−3.61 ± 0.79
Li^{+} [a]	−3.92 ± 0.56	−4.77 ± 0.76	−3.69 ± 0.02	−4.62 ± 0.08	−4.15 ± 0.12	−5.18 ± 0.23	−3.57 ± 0.11	−4.70 ± 0.09	−3.12 ± 0.22	−4.26 ± 0.18	−3.67 ± 0.76	−4.63 ± 1.13
Na^{+} [a]	−3.54 ± 0.12	−4.41 ± 0.15	−3.61 ± 0.11	−4.27 ± 0.21	−3.85 ± 0.25	−3.87 ± 0.69	−3.48 ± 0.19	−3.88 ± 0.11	−2.89 ± 0.17	−3.96 ± 0.13	−1.89 ± 0.64	−2.46 ± 0.82
NH_4^{+} [a]	−3.55 ± 0.20	−3.80 ± 0.01	−3.33 ± 0.11	−4.36 ± 0.50	−3.64 ± 0.11	−3.89 ± 0.08	−2.99 ± 0.27	−3.55 ± 0.12	−2.91 ± 0.17	−3.89 ± 0.11	−1.82 ± 0.65	−2.32 ± 0.79
K^{+} [a]	−2.63 ± 0.14	−3.73 ± 0.24	−3.08 ± 0.79	−3.37 ± 0.20	−2.24 ± 0.02	−3.63 ± 0.15	−2.50 ± 0.11	−3.34 ± 0.14	−2.59 ± 0.08	−3.51 ± 0.06	−1.07 ± 0.49	−1.85 ± 0.68
Phenylethylamine [a]	−1.27 ± 0.27	−2.31 ± 0.43	−1.00 ± 0.04	−2.41 ± 0.12	−1.21 ± 0.24	−2.44 ± 0.08	−1.12 ± 0.04	−2.53 ± 0.16	−1.60 ± 0.10	−2.52 ± 0.40	−0.37 ± 0.09	−0.92 ± 0.36
Epinephrine [a]	−0.65 ± 0.13	−0.68 ± 0.16	−0.57 ± 0.06	−0.73 ± 0.03	−0.55 ± 0.17	−0.61 ± 0.18	−0.60 ± 0.05	−0.73 ± 0.03	−0.71 ± 0.10	−0.48 ± 0.31	−0.11 ± 0.05	−0.09 ± 0.03
R-enantiomer [b]	0.06 ± 0.09	−0.03 ± 0.04	0.04 ± 0.12	0.05 ± 0.01	0.09 ± 0.01	−0.06 ± 0.04	0.16 ± 0.02	−0.15 ± 0.07	0.06 ± 0.04	0.00 ± 0.01	0.10 ± 0.08	0.15 ± 0.09

[a] Results obtained by separate solution method [52] at two concentration levels of concentration of interferent and primary ion: 1×10^{-4} mol L^{-1} and 1×10^{-3} mol L^{-1}. [b] Results obtained by mixed solution method (two solution method) [52] when the concentration of the pure solution of the primary ion (*S*-enantiomer) was 1×10^{-4} or 1×10^{-3} mol L^{-1} and the concentrations of the mixed solution containing the primary and the interfering ion were 1×10^{-4} or 1×10^{-3} mol L^{-1} in S and *R*-enantiomers, respectively.

spiked urine and synthetic serum). Direct potentiometric and standard addition methodologies were used for OFLX measurement in the mentioned matrices.

4.1 Determination of Ofloxacin in Pharmaceutical Preparations

Several pharmaceutical preparations with different OFLX or LVFX (the *S*-enantiomer) doses were analysed. Appropriate amounts of 10 powdered tablets were dissolved in buffer solution. For each pharmaceutical, three solutions with final OFLX/LVFX concentration from 5×10^{-5} to 3×10^{-4} mol L^{-1} were prepared and analysed by direct potentiometry. Results obtained were compared to those obtained by the USP reference method [53] (Table 4). Application of the Student's *t*-paired test indicates the absence of statistical differences as the calculated *t* value is 1.000 lower than the 2.571 (the theoretical *t* value) at a confidence level of 95%. Recoveries assays were also carried out in all the sample solutions previously examined. Recoveries rates between 99–107% were always obtained (Table 4). This emphasizes the noninterference of other species present in commercial formulations and corroborates data obtained by direct potentiometry.

4.2 Determination of Ofloxacin in Urine Sample

Analysis of species present in physiological matrices without preliminary treatment is an important feature often related to electroanalytical methodologies.

OFLX is usually oral administered in daily doses of 200–400 mg. The urinary concentrations are dose-dependent, but the percentage of the dose excreted via the kidneys remains approximately constant, 80% or more of the dose is recovered as unchanged OFLX. In a collection period of 6 hours following the administration of a single 200 mg and 400 mg oral dose of OFLX, the urine average concentration of OFLX is approximately 220 μg/mL and 427 μg/mL, respectively [9,10].

Known amounts of drug were added to blank urine aliquots to include the physiological concentrations obtained after the usual therapeutic dose, 200–600 μg mL^{-1} (5×10^{-4}–2×10^{-3} M). These samples were subsequently diluted in buffer solution for ionic strength and pH adjustments. Results obtained for the spiked urine samples are presented in Table 5. If spiked urine samples analysed contained at least 4×10^{-5} mol L^{-1}, between 99 to 104% of the OFLX added to the blank urine samples was encountered by direct potentiometric measurements, confirming the good selectivity of the method and indicating that the constituents of the human urine samples do not interfere.

However, results obtained by direct potentiometry procedure for spiked urine samples having ofloxacin concentrations below 4×10^{-5} mol L^{-1} presented inadequate relative deviations (values ranging from 10–20%) with a clear trend for higher values the lower the concentration

Table 4. Determination of OFLX or LVFX in pharmaceuticals by direct potentiometry and recovery assay results of the examined pharmaceuticals.

Preparation	Initial concentration ($mol\,L^{-1}$)	Direct potentiometry			Recovery study	
		Present method (g/tablet) [a]	Reference method (g/tablet) [b]	*RD* (%) [c]	Concentration added ($mol\,L^{-1}$)	Recovery (%) [d]
Tavanic	8.44×10^{-5}	0.497 ± 0.006	0.495 ± 0.004	0.20	1.31×10^{-4}	100.4 ± 0.9
	1.69×10^{-4}	0.501 ± 0.007		1.21	3.27×10^{-4}	104.1 ± 0.6
	2.53×10^{-4}	0.507 ± 0.004		2.42	4.37×10^{-4}	103.5 ± 0.5
Loxadin	8.43×10^{-5}	0.490 ± 0.002	0.513 ± 0.012	−4.48	1.31×10^{-4}	105.6 ± 0.3
	1.69×10^{-4}	0.501 ± 0.006		−2.34	3.27×10^{-4}	107.2 ± 0.9
	2.53×10^{-4}	0.492 ± 0.008		−4.09	4.37×10^{-4}	106.2 ± 0.8
J Neves	8.57×10^{-5}	0.509 ± 0.004	0.499 ± 0.009	2.00	1.31×10^{-4}	105.2 ± 0.2
	1.71×10^{-4}	0.506 ± 0.013		1.40	3.27×10^{-4}	106.9 ± 0.1
	2.53×10^{-4}	0.484 ± 0.002		−3.01	4.37×10^{-4}	106.2 ± 0.8
Bioquil	5.04×10^{-5}	0.398 ± 0.009	0.395 ± 0.004	−0.76	1.21×10^{-4}	102.4 ± 0.6
	1.01×10^{-4}	0.408 ± 0.016		3.29	3.02×10^{-4}	105.4 ± 0.1
	1.51×10^{-4}	0.415 ± 0.014		5.06	4.03×10^{-4}	105.9 ± 0.7
Ofloxacina Merck	5.04×10^{-5}	0.199 ± 0.002	0.195 ± 0.008	2.05	1.21×10^{-4}	104.6 ± 0.9
	1.01×10^{-4}	0.196 ± 0.001		0.51	3.02×10^{-4}	104.6 ± 1.1
	1.51×10^{-4}	0.197 ± 0.002		1.03	4.03×10^{-4}	100.2 ± 1.2
Tarivid	5.04×10^{-5}	0.195 ± 0.007	0.201 ± 0.006	−2.99	1.21×10^{-4}	103.2 ± 0.6
	1.01×10^{-4}	0.199 ± 0.004		−1.00	3.02×10^{-4}	102.7 ± 1.1
	1.51×10^{-4}	0.203 ± 0.003		1.00	4.03×10^{-4}	98.6 ± 1.9

[a] Average of 4 determinations ± standard deviation (*SD*) with four different units obtained by the proposed methodology. [b] Results obtained by a reference HPLC method presented as average of 3 determinations ± standard deviation (*SD*). [c] Relative deviation for the reference method content versus concentrations obtained by the present method. [d] Mean recovery values of 4 determinations ± standard deviation

Table 5. etermination of OFLX in urine samples by direct potentiometry and by the standard addition method.

Sample dilution	Added ($mol\,L^{-1}$)	Proposed method [a] ($mol\,L^{-1}$)	*RD* [b] (%)
Direct potentiometry			
1:25	4.01×10^{-5}	$4.12\times10^{-5}\pm1\times10^{-6}$	2.74
	5.99×10^{-5}	$6.07\times10^{-5}\pm9\times10^{-7}$	1.34
	6.99×10^{-5}	$7.24\times10^{-5}\pm1\times10^{-6}$	3.58
1:10	6.99×10^{-5}	$7.26\times10^{-5}\pm2\times10^{-6}$	3.86
	1.00×10^{-4}	$9.96\times10^{-5}\pm3\times10^{-6}$	−0.40
	1.40×10^{-4}	$1.41\times10^{-4}\pm4\times10^{-6}$	0.71
	1.49×10^{-4}	$1.49\times10^{-4}\pm3\times10^{-6}$	0.00
Standard addition method			
1:25	2.01×10^{-5}	$2.04\times10^{-5}\pm2\times10^{-7}$	1.49
	3.01×10^{-5}	$3.21\times10^{-5}\pm2\times10^{-8}$	6.64
	4.01×10^{-5}	$4.19\times10^{-5}\pm1\times10^{-7}$	4.49
1:10	4.99×10^{-5}	$4.98\times10^{-5}\pm3\times10^{-7}$	−0.20
	1.01×10^{-4}	$1.00\times10^{-4}\pm5\times10^{-7}$	−0.99
	1.97×10^{-4}	$1.96\times10^{-4}\pm2\times10^{-7}$	−0.51

[a] Average of 4 determinations ± standard deviation (*SD*) with four different units. [b] Relative deviation between the added content versus concentrations obtained by the present method.

tested. Problems due to ionic strength and unknown liquid junction potentials can play a significant role and be a major source of errors in the determination of OFLX activity at such low levels. The use of the standard additions method for OFLX determination in these samples enables to overcome such problems Furthermore the additions of OFLX to the sample lead to a higher OFLX/matrice ratios and therefore minimize the effect of interfering species in the sensor system (when the concentration of the primary ion is reduced the effect of interfering species is enlarged). As can be seen from Table 5, excellent results were obtained using this procedure, even at very low OFLX levels (relative deviations lower than 7% were obtained in all trials).

4.3 Determination of Ofloxacin in Serum

OFLX plasma concentrations increase dose-dependently with maximum serum concentrations being achieved one to two hours after an oral dose. Mean peak serum con-

Table 6. etermination of OFLX in synthetic serum sample by direct potentiometry and by the standard addition method.

Sample dilution	Added ($mol\,L^{-1}$)	Proposed method ($mol\,L^{-1}$) [a]	*RD* (%) [b]
Direct potentiometry			
1:2	4.00×10^{-6}	$4.08\times10^{-6}\pm1\times10^{-7}$	2.00
	6.01×10^{-6}	$5.78\times10^{-6}\pm2\times10^{-7}$	−3.83
	8.02×10^{-6}	$7.54\times10^{-6}\pm3\times10^{-7}$	−5.99
Standard addition method			
1:2	2.00×10^{-6}	$2.01\times10^{-6}\pm3\times10^{-8}$	0.50
	4.00×10^{-6}	$3.98\times10^{-6}\pm5\times10^{-8}$	−0.50
	8.09×10^{-6}	$8.39\times10^{-6}\pm1\times10^{-7}$	3.71
	1.01×10^{-5}	$1.03\times10^{-5}\pm4\times10^{-7}$	1.98

[a] Average of 4 determinations ± standard deviation (*SD*) with four different units. [b] Relative deviation between the added content versus concentrations obtained by the present method.

centration after single oral doses of 200, 300 or 400 mg of OFLX are 1.5 μg/mL, 2.4 μg/mL and 2.9 μg/mL, respectively. After multiple-dose administration of 200, 300 or 400 mg doses, peak serum levels of 2.2, 3.6 and 4.6 μg/mL, respectively, are predicted at steady state [9,10]. OFLX levels representative of the real situation were added to a synthetic serum sample (2–7 $\mu g\,mL^{-1}$; 6×10^{-6}–2×10^{-5} M) and analyte determinations were carried out after a 1:2 dilution with buffer solution to adjust ionic strength and pH. Results of these assays are shown in Table 6. For analyte concentration levels higher than $4\times10^{-6}\,mol\,L^{-1}$ adequate results were obtained by direct potentiometric measurements. In fact, relative deviations between the amount of OFLX added to blank serum sample and the one obtained by direct interpolation in the analytical curve were lower than 6%. OFLX concentrations lower than $4\times10^{-6}\,mol\,L^{-1}$ were determined by the standard addition method (Table 6). Relative deviations lower than 5% were obtained, This stresses the ability of the proposed sensor to determine low analyte concentrations in serum samples.

5 Conclusions

The proposed OFLX ion-selective electrode, based on tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ion exchanger immobilized in a PVC-matrix membrane, show high sensitivity (slope of 55 mV $decade^{-1}$), a wide working range (3×10^{-6} to $1\times10^{-2}\,mol\,L^{-1}$) and has a practical detection limit of $1\times10^{-6}\,mol\,L^{-1}$. The electrode can be used over de pH range of 1.1–5.6 without any significant pH effect. The electrode has a rapid response (< 10 s), exhibits long lifetime and good selectivity being an advantageous alternative for the quality control, routine analysis and monitoring of OFLX in pharmaceutical preparations and biological fluids. The procedure avoids the usual pretreatment steps necessary for OFLX assays in complex matrices and provides a simple, fast, accurate and precise method for drug determination in pharmaceuticals, human urine and serum samples. Results obtained stress out the fact that no interference from main inorganic ions and common excipients present in commercial preparations and biological matrices was found. Additionally, the proposed method presents some general advantages over common chromatographic and spectrophotometric procedures: it makes use of less sophisticated equipment (therefore being easier to operate and providing lower cost of analysis) and surpasses colour and turbidity problems associated with the suspensions and colloids formed during drug dissolution.

References

[1] L. S. Goodman, A. Gilman, L. L. Brunton, J. S. Lazo, K. L. Parker, *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York **2006**.

[2] W. Martindale, *Martindale: The Extra Pharmacopoeia*, Pharmaceutical Press, London **1996**.

[3] F. Cañada-Cañada, A. Espinosa-Mansilla, A. M. Peña, *J. Sep. Sci.* **2007**, *30*, 1242.

[4] W. Y. Sun, W. Y. Liu, L. B. Qu, *Chin. Chem. Lett.* **2007**, *18*, 1107.

[5] V. Andreu, C. Blasco, Y. Picó, *Trends Anal. Chem.* **2007**, *26*, 534.

[6] I. Hayakawa, S. Atarashi, S. Yokohama, M. Imamura, K. Sakano, M. Furukawa, *Antimicrob. Agents Chemother.* **1986**, *29*, 163.

[7] I. Morrissey, K. Hoshino, K. Sato, A. Yoshida, I. Hayakawa, M. G. Bures, L. L. Shen, *Antimicrob. Agents Chemother.* **1996**, *40*, 1775.

[8] T. Fujimoto, S. Mitsuhashi, *Chemotherapy* **1990**, *36*, 268.

[9] *Floxin (Ofloxacin) Drug Information: Uses, Side Effects, Drug Interactions and Warnings at RxList*, available at: http://www.rxlist.com/floxin-drug.htm.

[10] C. K. Naber, M. Hammer, M. Kinzig-Schippers, C. Sauber, F. Sörgel, E. A. Bygate, A. J. Fairless, K. Machka, K. G. Naber, *Antimicrob. Agents Chemother.* **2001**, *45*, 3524.

[11] L. Zivanovic, G. Zigic, M. Zecevic, *J. Chromatogr. A* **2006**, *1119*, 224–230.

[12] A. Espinosa-Mansilla, A. M. Peña, D. G. Gómez, F. S. López, *Talanta* **2006**, *68*, 1215.

[13] O. Ballesteros, I. Toro, V. Sanz-Nebot, A. Navalón, J. L. Vílchez, J. Barbosa, *J. Chromatogr. B* **2003**, *798*, 137.

[14] J. Tuerk, M. Reinders, D. Dreyer, T. K. Kiffmeyer, K. G. Schmidt, H. Kuss, *J. Chromatogr. B* **2006**, *831*, 72.

[15] C. Horstkötter, G. Blaschke, *J. Chromatogr. B* **2001**, *754*, 169.
[16] B. Awadallah, P. C. Schmidt, M. A. Wahl, *J. Chromatogr. A* **2003**, *988*, 135.
[17] H. Sun, A. Hao, Y. Yang, C. Zhu, J. Li, J. Shen, M. Yin, *J. Incl. Phenom. Macrocycl. Chem.* **2009**, *65*, 427.
[18] T. Arai, N. Nimura, T. Kinoshita, *J. Chromatogr. A* **1996**, *736*, 303.
[19] T. D. Boer, R. Mol, R. A.D. Zeeuw, G. J.D. Jong, K. Ensing, *Electrophoresis* **2001**, *22*, 1413.
[20] A. A. Elbashir, B. Saad, A. S.M. Ali, M. I. Saleh, H. Y. Aboul-Enein, *J. Liquid Chromatogr. Related Technol.* **2008**, *31*, 348.
[21] V. Kapetanovic, L. Milovanovic, M. Erceg, *Talanta* **1996**, *43*, 2123.
[22] S. Ashour, R. Al-Khalil, *Il Farmaco* **2005**, *60*, 771.
[23] A. Espinosa-Mansilla, A. M. Peña, F. Cañada-Cañada, D. G. Gómez, *Anal. Biochem.* **2005**, *347*, 275.
[24] D. Fang, H. Wu, Y. Ding, L. Hu, A. Xia, R. Yu, *Talanta* **2006**, *70*, 58.
[25] A. M. Peña, A. E. Mansilla, D. G. Gómez, A. C. Olivieri, H. C. Goicoechea, *Anal. Chem.* **2003**, *75*, 2640.
[26] O. Ballesteros, J. L. Vílchez, A. Navalón, *J. Pharm. Biomed. Anal.* **2002**, *30*, 1103.
[27] P. S. Francis, J. L. Adcock, *Anal. Chi. Acta* **2005**, *541*, 3.
[28] B. Li, Z. Zhang, L. Zhao, C. Xu, *Talanta* **2002**, *57*, 765.
[29] F. A. Aly, S. A. Al-Tamimi, A. A. Alwarthan, *Talanta* **2001**, *53*, 885.
[30] Y. Ni, Y. Wang, S. Kokot, *Talanta* **2006**, *69*, 216.
[31] G. Zhou, J. Pan, *Anal. Chim. Acta* **1995**, *307*, 49.
[32] A. Tamer, *Anal. Chim. Acta* **1990**, *231*, 129.
[33] W. Y. Sun, W. Y. Liu, L. B. Qu, *Chin. Chem. Lett.* **2007**, *18*, 1107.
[34] R. Stefan, G. E. Baiulescu, H. Y. Aboul-Enien, *Crit. Rev. Anal. Chem.* **1997**, *27*, 307.
[35] M. S. Frant, *Analyst* **1994**, *119*, 2293.
[36] A. Lewenstam, M. Maj-Zurawska, A. Hulanicki, *Electroanalysis* **1991**, *3*, 727.
[37] M. Giahi, M. Arvand, M. Mirzaei, M. A. Bagherinia, *Anal. Lett.* **2009**, *42*, 870.
[38] M. S. García, J. Ortuño, M. I. Albero, M. Cuartero, *Anal. Bioanal. Chem.* **2009**, *394*, 1563.
[39] P. Buhlmann, E. Pretsch, E. Bakker, *Chem. Rev.* **1998**, *98*, 1593.
[40] E. Bakker, P. Buhlmann, E. Pretsch, *Chem. Rev.* **1997**, *97*, 3083.
[41] G. D. Christian, *Analytical Chemistry,* 6th ed., Wiley, Chichester **2009**.
[42] R. Lapa, J. Lima, A. Roque da Silva, *Il Farmaco* **1990**, *45*, 901.
[43] A. R. Pires, A. N. Araújo, M. C. Montenegro, P. Chocholous, P. Solich, *J. Pharm. Biomed. Anal.* **2008**, *46*, 683.
[44] A. Ferancová, J. Labuda, *Fresenius J. Anal. Chem.* **2001**, *370*, 1.
[45] B. Awadallah, P. C. Schmidt, M. A. Wahl, *J. Chromatogr. A* **2003**, *988*, 135.
[46] P. S. Bates, R. Kataky, D. Parker, *Analyst* **1994**, *119*, 181.
[47] E. Bakker, E. Pretsch, *Anal. Chim. Acta* **1995**, *309*, 7.
[48] S. S. Hassan, W. H. Mahmoud, M. A. Elmosallamy, A. H. Othman, *Anal. Chim. Acta* **1999**, *378*, 299.
[49] J. H. Khorasani, M. K. Amini, H. Motaghi, S. Tangestaninejad, M. Moghadam, *Sens. Actuators B. Chem.* **2002**, *87*, 448.
[50] T. Masadome, S. Wakida, Y. Kawabata, T. Imato, N. Ishibashi, *Anal. Sci.* **1992**, *8*, 89.
[51] R. P. Buck, E. Lindner, *Pure Appl. Chem.* **1994**, *66*, 2527.
[52] Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda, S. Amemiya, *Pure Appl. Chem.* **2000**, *72*, 1851.
[53] *U. S. Pharmacopoeia 30 National Formulary 25*, The United States Pharmacopeia Convention Inc. **2007**.

