Ofloxacin Determination in Urine, Serum and Pharmaceuticals Using an Automatic Flow Potentiometric System

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An automatic system was developed to determine ofloxacin in biological fluids and pharmaceutical formulations. Drug detection was carried out by a potentiometric membrane sensor based on [bis(trifluoromethyl)phenyl]borate as molecular-recognition material. The tubular shaped detector system was solidly attached to the manifold, creating a high-throughput stable setup (50 samples per hour) appropriate for routine antibiotic assessment. Under the optimized flow conditions, the sensor displayed a mean detection limit of 1×10^{-5} M, a linear response over the concentrations of 2×10^{-5} to 5×10^{-3} M (slope of 57.4 mV decade^{-1}) and a wide working pH range (2.1 – 6.6). The procedure was successfully applied to ofloxacin analysis in pharmaceuticals (relative deviation lower than 6%) and biological fluids at levels usually found after drug administration of clinical doses (recoveries between 91 and 106%). No significant interference from common excipients found in commercial formulations and inorganic ions usually present in biological fluids was noticed.

Keywords Automatic determination, ofloxacin, pharmaceuticals, serum, urine

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Introduction

Ofloxacin (OFLX) [(±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxacine-6-carboxylic acid] (Fig. 1) is a synthetic fluoroquinolone antibiotic active against both Gram-positive and Gram-negative organisms, as well as intracellular pathogens responsible for atypical pneumonia.[1] Its bactericidal effect is achieved through binding to DNA gyrase, and subsequent inhibition of bacterial DNA replication and transcription. Due to its broad-spectrum activity, good absorption and low frequency of adverse effects, it is commonly prescribed in the clinical treatment of skin, respiratory, urinary and gastrointestinal tract infections.[1,2] The drug is marketed as a racemic mixture (consisting of equal amounts of the enantiomers) or as levofloxacin (LVFX), the (–)-(S)-enantiomer, which is the more active isomer.[3]

According to literature, the usual therapeutic dose of ofloxacin tablets is 200 to 500 mg every 12 h.[2-4] Following oral administration, the bioavailability is approximately 98% and the peak serum levels between 2.2 – 4.6 μg mL^{-1} are usually attained one to two hours after dosing. The drug undergoes limited metabolism, and elimination is mainly by renal excretion; between 65 and 80% of an administered oral dose of OFLX is excreted through the kidneys within 48 h of dosing in an unaltered form. The final concentrations in urine of treated patients are in the range of 200 to 420 μg mL^{-1}.

Quality control carried out by the pharmaceutical industry involves the determination of multiple parameters for both raw materials and end products. Reliable and specific assays are required to ensure the safety, efficacy and quality of pharmaceutical products. Several methods have been reported for the determination of OFLX in pure form, dosage forms and in biological fluids. Most of the analytical methods used are chromatographic,[5-8] including the official USP procedure for the determination of OFLX in dosage forms.[9] Despite their high sensitivities, chromatographic methodologies are

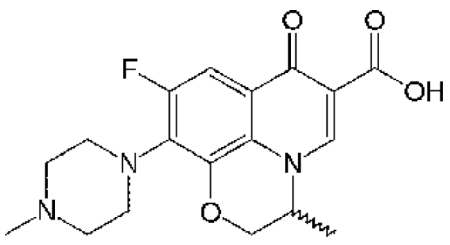

Fig. 1 Chemical structure of ofloxacin.

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time-consuming, expensive and involve the use of complex procedures with several sample manipulations before injection into the system.

Alternatively, chemiluminescence (CL) methods, based on reactions with acidic tris(2,2′-bipyridyl)ruthenium(III) and Ce(IV),[10] or an enhancement of the emission from either the oxidation of sulfite[11-14] or the reaction with sodium nitrite and hydrogen peroxide, have also been proposed.[15] In order to obtain results with adequate accuracy and precision, the amounts of CL reagents must be rigorously controlled, a process that presents some difficulty when using conventional methodologies. To overcome these drawbacks, CL methods were linked to flow-injection analysis (FIA), with clear advantages over conventional procedures since they enable accurate determinations of OFLX in different matrices, with considerable reductions in the analysis time, precise control of volumes and decrease in reagents consumption.[16] Nevertheless, these systems are still complex, containing several flow lines to accommodate, deliver and merge all reagents. Additionally, sample pretreatment steps (namely filtration and centrifugation) are often necessary, and despite the large reduction in reagents consumption, the assays are expensive. FIA coupled to potentiometric detection based on ion-selective electrodes (ISEs) have also been widely exploited in several areas, including pharmaceutical and clinical analysis. The low cost, adequate selectivity and accuracy, wide linear concentration range (with limited or no sample preparation steps) and relatively short response times explain their widespread use.[17-21] Additionally, some of ISE's performance characteristics improve when electrodes are used in flow systems: the permanent liquid stream has a conditioning effect on the sensor membrane while improving sensitivity, stability and reproducibility. In this work, a single-line flow-through potentiometric system for the determination of OFLX in pharmaceuticals and biological fluids is described. A tubular-shaped ion-selective electrode, with a sensor system composed by a mixture of potassium tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate dissolved in 2-nitrophenyloctyl ether immobilized in PVC was used as an indicator electrode.[22] Tubular structures were solidly attached to the manifold, creating a stable setup, which was easily handled and free of mechanical problems. In this way, the aforementioned intrinsic advantages of potentiometric procedures were enhanced by its incorporation in the flow line, giving rise to increased measurements stability, repeatability and sample throughput.

Experimental

Reagents and chemicals

Deionized water (with a specific conductance lower than 0.1 μS cm^{-1}) and analytical-grade chemicals without additional purification were used. The following reagents were used for ISEs preparation: high-molecular-weight poly(vinyl chloride) (PVC, Fluka 81392), potassium tetrakis[3,5-bis(trifluoromethyl) phenyl]borate (KTFPB, Fluka 60588), 2-nitrophenyloctyl ether (ONPOE, Fluka-73732) and tetrahydrofuran (THF, Sigma-Aldrich 34865). A 0.1 M NaH_2PO_4 solution (with the pH adjusted to 2.5) was used for ionic strength and pH control. Standard 1×10^{-2} M OFLX (Sigma O8757) or LVFX (Fluka 28266) solutions were prepared daily by rigorous weighing of the solid and dilution in the buffer solution. Working solutions (2×10^{-5} – 5×10^{-3} M) were prepared by appropriate dilutions of these OFLX/LVFX standards with a phosphate buffer. A low OFLX concentration (10^{-6} M) was always included in the buffer carrier stream to assure a constant conditioning of the electrode surface, and to contribute to baseline stabilization. All ion inorganic interferent standard solutions were prepared with analytical-grade chloride salts. Commercial dosage tablets containing OFLX (Tarivid® 200 mg, Bioquil® 400 mg and Ofloxacina Merck® 200 mg) or LVFX (Tavanic® 500 mg, Loxadin® 500 mg and Levofloxacina J Neves® 500 mg) were purchased from local drug stores. Drug-free human urine samples used in this study were collected from 10 healthy volunteers. Synthetic serum sample contained 138 mM NaCl, 2.8 mM KCl, 1.3 mM KH_2PO_4, 2.5 mM $CaCl_2{\cdot}2H_2O$, 1.0 mM $MgSO_4{\cdot}7H_2O$ and 6% bovine serum albumin.[23] Urine and serum samples were spiked with appropriate amounts of analyte, to reproduce biological concentrations usually found in these fluids. Sample analyses according to the USP method[9] were carried out in a chromatographic Hewlett Packard (HP) Agilent Series 1100 system, made up of a HP pump (Model G1311A), connected to a Rheodyne injector Model 7725i (20 μL loop) and a Merck LichroCART RP 18 column (4 × 250 mm) packed with 5 μm beads, and a HP diode-array detector (Model G1314). Data were processed using HP Chemstations software (Revision A. 07.01).

Membrane preparation and sensor construction

The selective membrane consisted of a KTFPB electroactive material dispersed in a PVC matrix of ONPOE plasticizer. Polymeric membranes were prepared by the dissolution of 10 mg of KTFPB in 180 mg of ONPOE, followed by the addition of 80 mg of powdered PVC in 3 mL of THF. This solution was then added dropwise on a channel drilled longitudinally in a perspex tubular conductor support, made up of a mixture of Araldite epoxy resin and with graphite.[24] After THF evaporation, several applications of the sensor solution were carried out until a 1-mm thick membrane was obtained. In this way a flow channel of about 0.8 mm i.d. and 10 mm length was created (Fig. 2). Once the application of the sensor membrane was completed, the electrodes were left to dry at room temperature overnight for total evaporation of THF. Before use, the tubular electrodes were attached to the flow system, and conditioned in a 1×10^{-3} M OFLX solution at a flow rate of 1.0 mL min^{-1} for approximately 30 min.

Sample preparation

Procedure for the analysis of ofloxacin in pharmaceuticals. Ten tablets of each pharmaceutical were weighed and finely powdered. A part of the powder (equivalent to 150 mg of OFLX/LVFX) was accurately weighed, dissolved in the buffer solution and transferred into a 50-mL volumetric flask. Each of these original solutions was further diluted to obtain working solutions with three different dilution levels of 1:100, 1:50 and 1:30. Potentiometric measurements were next carried out following the procedure described below, and drug content was determined by direct potentiometry.

Procedures for the analysis of ofloxacin in urine and serum. Human urine and serum were first spiked with different amounts of OFLX 1×10^{-2} M standard solution to meet active therapeutical concentrations: 5×10^{-4} to 2×10^{-3} M for urine and 6×10^{-6} to 2×10^{-5} M for serum.[2-4] Spiked samples were diluted with the buffer solution to adjust the ionic strength and the pH (1:25 or 1:10 for urine matrices and 1:2 for serum samples), and later supplemented with four known volumes of the drug standard solution. Volumes of 100, 200, 500 and 700 μL of a 1×10^{-2} M OFLX solution were added to 50.0 mL of diluted urine samples. For serum samples, 20.0 mL aliquots were spiked with 10, 100, 200 and 300 μL. The potentials of the solutions were recorded, and these data were used to

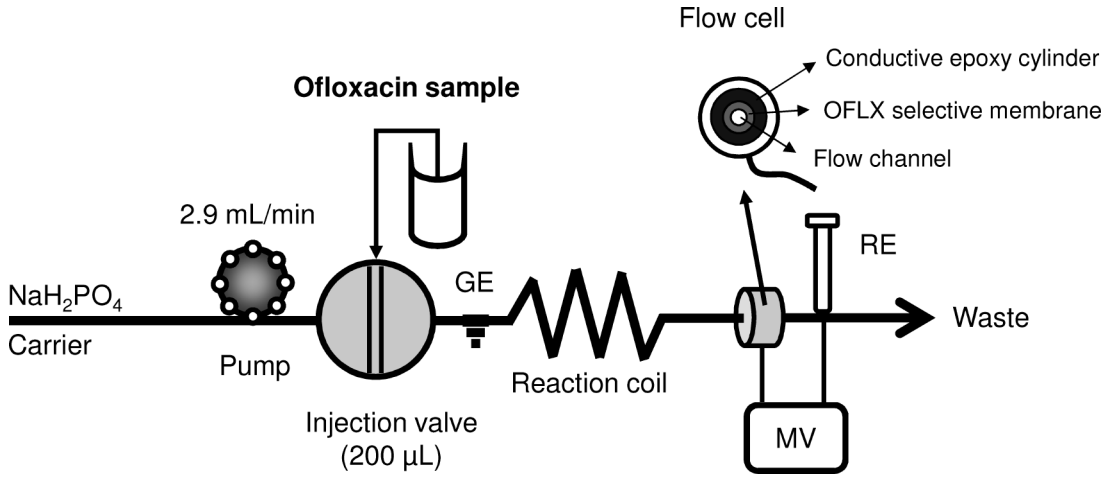

Fig. 2 Schematic diagram of the high-throughput flow system for ofloxacin determination; GE, ground electrode; RE, reference electrode; Flow cell, ion selective electrode.

calculate the concentration of OFLX in each sample solution, by the standard addition technique.

Procedures for the analysis of ofloxacin by HPLC. A validated method for the determination of OFLX in pharmaceuticals was used.[9] The analysis was performed using a mobile phase composed of a mixture of sodium dodecyl sulfate (0.24% aqueous solution), acetonitrile and glacial acetic acid (580:400:20) filtered, degassed and at a flow rate of 1.5 mL min^{-1}. The UV detector was set at 294 nm. The preparation of pharmaceuticals sample solutions and OFLX standard solutions involved dissolution in 0.05 M hydrochloric acid of a sample amount corresponding to a final OFLX concentration of 0.06 g L^{-1}. These solutions were subsequently filtered through a 0.5-µm filter. A calibration graph was constructed using standard OFLX solutions before analyzing the pharmaceutical samples.

Apparatus and measurements

FIA measurements were performed using a single-stream manifold (Fig. 2). The flow setup include a Minipuls 3 Gilson (Viliers-le-Bell, France) four-channel peristaltic pump with a PVC pumping tube (1.3 mm i.d.) of the same brand and an injection valve (Rheodyne, Model 5020) with an exchangeable sample loop.

A Crison micropH, Model 2002 voltmeter (±0.1 mV sensitivity) was used to measure the potential differences of the electrochemical cell: a double junction Orion (Model 900200) Ag/AgCl reference electrode (the outer compartment of the reference electrode contained the buffer solution) and a home-made tubular shaped OFLX-selective electrode, with a graphite-based electrical contact and no inner reference. The decimilivoltammeter was interfaced to a strip chart recorder, Model BD111 from Kipp and Zonn (Deflt, Netherlands). A 1-cm long homemade stainless-steel tube (0.8 mm i.d.) ground electrode was included in the manifold to minimize the system electrical background noise. The 35 cm reaction coil (RC) and flow lines were made with i.d. 0.8 mm PTFE tubing. The pH measurements were performed with a WTW Model SenTix 41 glass electrode.

To assess the general tubular electrode characteristics (namely, the practical detection limit and the lower limit of linear response), a series of freshly prepared 2×10^{-6} to 1×10^{-2} M standard OFLX solutions were injected (200 µL) into the buffer flow stream at a flow rate of 2.9 mL min^{-1}, and a calibration curve was constructed by plotting the average peak potential of the signals for each standard (injected in triplicate) *versus* log[$OFLX^+$].

Results and Discussion

Study of the experimental variables

Preliminary studies were performed to optimize the essentials flow variables: the sample volume, flow rate and reactor length, in order to obtain adequate sensitivity, sampling rate and concentration working range under low-dispersion conditions. In a potentiometric flow apparatus, the measurement precision depends strongly on the maintenance of constant hydrodynamic conditions and minimization of the sample dispersion. To achieve such purposes, a low-dispersion single-channel flow manifold was adopted (Fig. 2). The sample was injected into a buffer stream, and the presence of OFLX led to an increase in the analytical signal. The effect of the sample size and the flow rate on the analytical response was studied with a set of OFLX standard solutions. The results obtained for sample volumes in the range of 50 to 1000 µL showed that lower volumes led to measurements with reduced precision, while higher volumes produced long baseline return times. A 200-µL sample volume was chosen as a compromise solution between adequate precision and the sampling rate. Regarding the flow rate, no significant differences between the results obtained with flow rates from 0.8 up to 5.7 mL min^{-1} were encountered. However, at high pumping rates, the signals became more unstable, the carrier consumption increased and the sensitivity slightly decreased (±1 mV/decade of concentration). On the other hand, low pumping rates made the analysis time-consuming. On balance, the carrier flow rate was set at 2.9 mL min^{-1}. Experiments to establish the influence of the reactor length were next carried out. Lengths from 20 to 60 cm were tested. The obtained results showed an increase in the signal intensity and the stability for lengths of up to 35 cm. Therefore, a 35-cm reactor was selected to minimize the sample dispersion, and consequently enhance the sensitivity. The effect of the pH on the electrode potential was studied over the pH range of 1 – 13 by adding small volumes of concentrated sulfuric acid or sodium hydroxide solutions to aqueous 5×10^{-4} and 5×10^{-3} M

OFLX solutions. Tracing of the potential/pH diagrams showed no significant change on the potential response over the pH range of 2.1 to 6.6 (differences lower than 5 mV were registered), taken as the operational pH range of the electrode. OFLX has zwitterionic properties by virtue of the carboxyl group being deprotonated at basic pH values (pK_{a1} = 6.0), and its amino function in position 4 of the piperazine ring being positively charged under acidic conditions (pK_{a2} = 8.0). At the pH interval mentioned above, the OFLX molecules were protonated, positively charged, and could be sensed by an anion-exchanger, KTFPB, present in the membrane. In solutions with pH higher than 6, significant fractions of OFLX ions changed to the corresponding deprotonated forms, which were not detected by the electrode, and a clear decrease in the recorded analytical signals was observed.

Analytical features

The above-described optimum flow conditions were used to determine the analytical figures of merit of the proposed methodology. The lower limit of linear response (LLLR) and the practical detection limit (PDL) were established from five independent calibration curves in the concentration interval from 2×10^{-6} to 1×10^{-2} M. Average values of 1.8×10^{-5} ($\pm 7 \times 10^{-6}$) M for the LLLR and 1.1×10^{-5} ($\pm 2 \times 10^{-6}$) M for the PDL were obtained (figures in brackets represent the standard deviation). Sensors displayed a wide range of linear response (2×10^{-5} to 5×10^{-3} M) with a regression equation of: $\Delta E = 286.6(\pm 10.5) + 57.4(\pm 2.4) \log [\text{OFLX}]$; $r^2 = 0.995(\pm 0.003)$ (n = 15). Relative standard deviations lower than 4% were obtained for the slope and intercept values pointing out adequate measurement precision. The flow system allowed a frequency of 50 samples h^{-1}; also, the response of ISE remained constant for at least four months, during which time no appreciable change in the calibration characteristics or response time was observed.

Interference studies

To assess the applicability of the system for antibiotic quantification in pharmaceuticals and biological samples, the effect of the main inorganic cations usually present in human fluids were investigated under the optimum experimental conditions. Being positively charged and existing in samples tested in concentrations much higher than that of the primary species, they could have a significant influence in the potential of the membrane. Analytical standards were injected into the flow system, and potentiometric selectivity coefficients ($\log K^{\text{pot}}_{\text{OFLX,I}}$) were determined, using the separate solutions method (SSM), at two concentration levels (1×10^{-4} and 1×10^{-3} M) of primary (OFLX) and interfering ion (I), according to IUPAC guidelines.[25] Potential values corresponding to the peak heights for the same drug and the interferent (I) concentrations were used to calculate $\log K^{\text{pot}}_{\text{OFLX,I}}$ by applying a rearranged Nicolsky equation:

$$\log K^{\text{pot}}_{\text{OFLX,I}} = \left(\frac{E_2 - E_2}{S}\right) + \left(1 - \frac{1}{z}\right) \times \log[\text{OFLX}], \quad (1)$$

where E_1 is the potential of the electrode in the OFLX solution and E_2 the potential of the electrode in the interferent solution (I) (at the same concentration level of primary ion); z the interferent species charge, and S the slope of the calibration graph. Small-sized selectivity coefficients were obtained for all inorganic species tested (Table 1), pointing out that none of these cations interfere even at concentrations higher than those usually found in biological fluids. Since OFLX is a racemic mixture, consisting of equal amounts of R- and S-enantiomers, $\log K^{\text{pot}}_{\text{S-isomer, R-isomer}}$ was also assessed. The S-enantiomer was considered to be the primary species, and the R-enantiomer the interferent ion. No chiral selectivity was observed, since the net charge of the enantiomers are equal, and the mechanism of action of these membranes is based on electrostatic interactions ($\log K^{\text{pot}}_{\text{S-isomer, R-isomer}}$).

Table 1 Potentiometric selectivity coefficients ($\log K^{\text{pot}}_{\text{OFLX,I}}$) of the ofloxacin electrode[a]

Inteferent ion (I)	1×10^{-4} M	1×10^{-3} M
Ca^{2+}[b]	–3.57 ± 0.05	–4.07 ± 0.02
Mg^{2+}[b]	–3.34 ± 0.01	–3.94 ± 0.003
Li^{+}[b]	–1.53 ± 0.004	–2.61 ± 0.01
NH_4^{+}[b]	–1.55 ± 0.01	–2.57 ± 0.04
K^{+}[b]	–1.50 ± 0.01	–2.52 ± 0.02
R-Enantiomer[c]	–0.09 ± 0.05	–0.07 ± 0.04

a. Mean ± standard deviation of 4 results obtained.
b. Results obtained by separate solution method at two concentration levels of interferent and primary ion: 1×10^{-4} M and 1×10^{-3} M.
c. Results obtained by mixed solution method (two solution method) when the concentration of the pure solution of the primary ion (S-enantiomer) was 1×10^{-4} or 1×10^{-3} M and the concentrations of the mixed solution containing the primary and the interfering ion were 1×10^{-4} or 1×10^{-3} M in S- and R-enantiomers, respectively.

The interference of some excipients and additives found in commercial pharmaceutical preparations was also examined. Solutions with a fixed amount of antibiotic (1×10^{-4} M), but with a different excipient (or additive)/OFLX ratio (w/w), were injected in the flow system and their signals compared to that obtained with a pure OFLX 1×10^{-4} M solution. The excipient/additive was considered to have influence when the relative error of the measurement was higher than 5%. None of the species tested, silicon dioxide, glucose, lactose, starch, magnesium stearate, polyethylene glycol and titanium, caused interference up to an excipient/ofloxacin ratio (w/w) of 100, a value much higher than that found in commercial formulations.

Several coordination compounds of Fe^{3+}, Al^{3+}, Cu^{2+} and Ni^{2+} with OFLX have been described.[26,27] Since ion-selective electrodes respond only to an uncomplexed analyte, the formation of such chelates would impair the use of potentiometric devices for drug analysis. To assess the influence of these metals in OFLX determination, synthetic mixtures with a constant level of OFLX (1×10^{-4} M) and increasing amounts of metals were prepared. No interference was observed for Ni^{2+} and Fe^{3+}, even at a molar metal/OFLX ratio of 100 fold. Cu^{2+} and Al^{3+} showed a negative interference at a molar ratio higher than 10 fold. Both ratios were higher than those found in all analyzed samples.

Analytical applications

The developed procedure was used to evaluate the drug concentration on different samples. Considering the amount of antibiotic expected in each of the matrices tested, direct potentiometric measurements were carried out for drug evaluation in pharmaceuticals, and the standard addition methodology was adopted when using biological fluids.

Analysis of pharmaceutical samples

Following the procedure described in the Experimental section, the apparatus was used to determine of OFLX (or LVFX) in pharmaceutical formulations. Each pharmaceutical

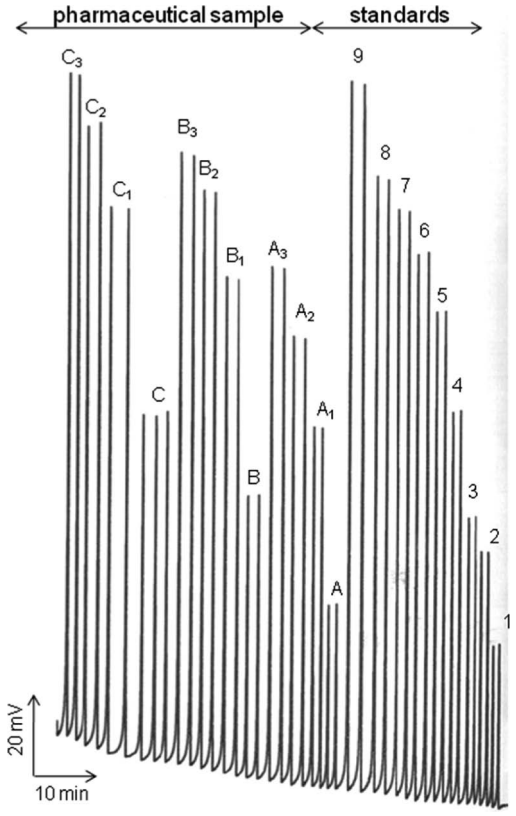

Fig. 3 Recording for ofloxacin determination in pharmaceuticals. Standard solutions: (1) 4×10^{-5}, (2) 8×10^{-5}, (3) 1×10^{-4}, (4) 2×10^{-4}, (5) 4×10^{-4}, (6) 6×10^{-4}, (7) 8×10^{-4}, (8) 1×10^{-3}, (9) 2×10^{-3} M. Pharmaceutical sample: A, B and C-100, 50 and 30-fold diluted pharmaceutical sample solution; A_1 – A_3, B_1 – B_3 and C_1 – C_3, additions carried out for the recovery assays.

sample solution prepared was analyzed with no further treatments. In every set of analysis, standard solutions were first injected to establish a linear relation of the average potential *versus* the logarithm of the concentration. Pharmaceutical drug concentrations were determined by interpolation of the analytical signals obtained in this graph. As described above, each original pharmaceutical solution was subjected to three different dilution levels (1:100, 1:50 and 1:30) in order to assess the precision of the procedure over the concentration working range. A recorder output of these determinations is shown in Fig. 3.

The obtained results are summarized in Table 2. The precision of the analytical signals was fully acceptable; in all instances relative standard deviations lower than 6% were obtained.

The application of the Student's *t*-paired test indicates the absence of any statistical differences between the results obtained by the USP reference method and those given by the proposed method, since the calculated *t* value was 0.784 lower than the 2.571 (the theoretical *t* value) at a confidence level of 95%.

Analysis of urine and serum samples

Known amounts of OFLX were added to drug-free urine and serum samples to meet therapeutical concentrations. These OFLX concentrations were lower than the LLLR, or too close to the lower limit of linearity. In this region the measurement precision is limited, and small changes in the analytical signal have a considerable effect on quantification, preventing analysis by direct potentiometry. Therefore, the standard addition method was used to measure the drug in these matrices. The results of these assays are given in Table 3. The values clearly confirm that the procedure is suitable for assessing OFLX in clinical samples containing low levels of analyte. Furthermore, the response of ISE remained constant, with stable values for both the slopes and potential readings, even when the membrane was exposed to biological matrices for a period of over 8 h.

Table 2 Determination of ofloxacin or levofloxacin in pharmaceuticals by direct potentiometry and results of recovery assays of the examined pharmaceuticals

Preparation	Dilution factor	Present method (g/tablet)[a]	Reference method (g/tablet)[b]	RD, %[c]
Tavanic	100	0.498 ± 0.012	0.495 ± 0.004	1.08
	50	0.514 ± 0.026		
	30	0.489 ± 0.022		
Loxadin	100	0.474 ± 0.031	0.513 ± 0.012	–2.92
	50	0.515 ± 0.018		
	30	0.505 ± 0.017		
J Neves	100	0.502 ± 0.018	0.499 ± 0.009	0.80
	50	0.504 ± 0.025		
	30	0.503 ± 0.033		
Bioquil	100	0.401 ± 0.011	0.395 ± 0.004	1.69
	50	0.398 ± 0.016		
	30	0.406 ± 0.018		
Ofloxacina Merck	100	0.203 ± 0.009	0.195 ± 0.008	6.15
	50	0.205 ± 0.011		
	30	0.213 ± 0.012		
Tarivid	100	0.196 ± 0.010	0.201 ± 0.006	–2.99
	50	0.186 ± 0.015		
	30	0.203 ± 0.016		

a. Mean of 3 determinations ± standard deviation (SD).
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* mean concentrations obtained by the flow procedure.

Table 3 Determination of ofloxacin in urine and serum samples by the standard addition method

Sample[a]	Added/M	Found/M[b]	RD, %[c]
Urine (1:10)	5.13×10^{-5}	$5.09 \times 10^{-5} \pm 1 \times 10^{-6}$	99.2
	6.00×10^{-5}	$6.12 \times 10^{-5} \pm 9 \times 10^{-7}$	102.0
	7.18×10^{-5}	$7.14 \times 10^{-5} \pm 1 \times 10^{-6}$	99.4
	1.00×10^{-4}	$9.79 \times 10^{-5} \pm 3 \times 10^{-6}$	97.9
	1.23×10^{-4}	$1.15 \times 10^{-4} \pm 3 \times 10^{-6}$	93.5
	1.40×10^{-4}	$1.32 \times 10^{-4} \pm 2 \times 10^{-6}$	94.3
Urine (1:25)	2.05×10^{-5}	$1.98 \times 10^{-5} \pm 2 \times 10^{-7}$	96.6
	3.08×10^{-5}	$3.27 \times 10^{-5} \pm 8 \times 10^{-7}$	106.2
	4.10×10^{-5}	$3.97 \times 10^{-5} \pm 1 \times 10^{-6}$	96.8
	6.00×10^{-5}	$6.20 \times 10^{-5} \pm 7 \times 10^{-7}$	103.3
	7.00×10^{-5}	$6.39 \times 10^{-5} \pm 2 \times 10^{-6}$	91.3
	1.00×10^{-4}	$9.79 \times 10^{-5} \pm 4 \times 10^{-6}$	97.9
Serum (1:2)	4.09×10^{-6}	$4.30 \times 10^{-6} \pm 5 \times 10^{-8}$	105.1
	6.13×10^{-6}	$6.34 \times 10^{-6} \pm 2 \times 10^{-7}$	103.4
	8.18×10^{-6}	$8.63 \times 10^{-6} \pm 1 \times 10^{-7}$	105.5
	1.02×10^{-5}	$9.98 \times 10^{-6} \pm 4 \times 10^{-7}$	97.8

a. In brackets are the dilution made in each sample before analysis.
b. Average of 3 determinations ± standard deviation.
c. Relative deviation between the concentration obtained by the present method *versus* the added content.

Conclusions

The flow potentiometric method developed proved to be an advantageous alternative to the time-consuming chromatographic and CL procedures. Good quality results, low sample consumption and a sample throughput of about 50 samples per hour were achieved using this procedure. The setup devised to accommodate the tubular shaped OFLX selective membrane was simple, containing only a single flow line and one peristaltic pump. The potentiometric sensor used was easily prepared, exhibited a long lifetime, and exhibited high sensivity. This tubular structure was solidly attached to the manifold, creating a stable system, free of mechanical problems and thereby appropriate for routine determinations. The time between putting the system into operation and the first determination was less than 5 min, including the time necessary to stabilize the baseline. After a simple dilution step, direct injection of the pharmaceutical mixtures and biological samples was feasible without any other pretreatment prior to the analysis. Adequate selectivity of the sensor was confirmed by drug recover values in biological fluids of 91 to 106%, and a relative deviation in commercial pharmaceutical formulations lower than 6%. The automatic flow system proposed here shows promising applicability for routine determinations and monitoring of OFLX in pharmaceuticals and clinical samples, and is advantageous over the tedious, expensive and slow official or chromatographic procedures methods previously described.

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