Egypt J. Anal. Chem., Vol. 21, PP. 81-92 (2012)

Determination of Levofloxacin and Lomefloxacin

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Summary: Levofloxacin (Le) or Lomefloxacin (Lo) is sensitively detected in pharmaceuticals by potentiometric method, based on the formation of an ion-pair complexes between levofloxacin or lomefloxacin cation and ammonium reinckate and flavinic acid. Two poly (vinyl chloride) conventional membrane sensors for each drug were fabricated, characterized, compared, and applied. The sensors exhibit linear response in the range of $1x10^{-2}$–$1x10^{-4}$ M with cationic slopes of 53.2, 51.4 and 50.3, 48.5 mV decade^{-1} at 25°C over the pH range 4-6, for lomefloxacin and levofloxacin sensors, respectively. Both drug electrodes show reasonable selectivity towards some related compounds and inorganic cations. The prepared electrodes are successfully applied to the determination of levofloxacin or lomefloxacin in bulk powder and pharmaceutical products. Results with mean accuracy 99.7 %, 99.5 % and 99.1, 99.0 for the nominal concentrations were obtained for levofloxacin and lomefloxacin, respectively, which compare well with data obtained using spectrophotometric (UV-Vis) method.

Introduction

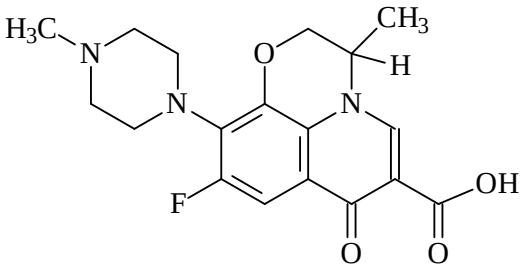
Flurorquinolones are a class of important synthetic antibiotics, which are active against both Gram (+) and Gram (-) bacteria through inhibition of their DNA gyrase.(1) Their excellent pharmacokinetic profile, good tissue penetration and wide scope of activity have made flurorquinolones very useful in human and veterinary medicine.(2) Levofloxacin (Figure, 1A) (9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7Hpyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate) is an antibacterial active against a broad spectrum of Gram-positive and Gram-negative microorganisms.(3) Levofloxacin is the pure (-)-(s)-enantiomer of the racemic drug substance. The mechanism of action of levofloxacin antimicrobials involves the inhibition of bacterial topoisomerase IV and DNA gyrase (both of which are type II topoismerases), enzymes required for DNA replication, transcription,

repair, and recombination. It is also active against causes of a typical respiratory infection such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.[4] Lomefloxacin (1-Ethyl-6+,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid) (Fig. 1 B) is often used for treatment of respiratory tract, urinary tract, skin and skin-structure infections.[5,6]

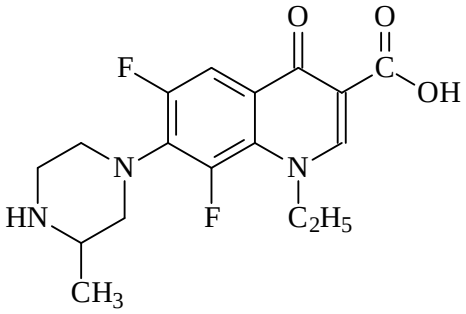
Due to the importance of using these fluoroquinolone drugs as medicines and the need for clinical and pharmacological study, a fast and sensitive analytical methods for determination of these drugs in biological fluids is required. The most common techniques used for determination of these drugs in biological fluids include high performance liquid chromatography (HPLC),[7-10] microbiological assay[11] capillary electrophoresis (CE),[12,13] chemiluminescene,[14-15] voltammetry,[16] polarography,[17] spectrophotometric[18-23], spectrofluorimetry,[24,25] atomic absorption spectrometry[26] and fluorimetric methods.[26]

In this paper, potentiometric membrane sensors for quantification of both levofloxacin and lomefloxacin drugs are described. The prepared sensors are based on the use of two ion exchanger (ammonium reineckate and flavinic acid) to prepare electroactive materials. These compounds is significantly enhances the selectivity of these sensors towards the primary drug ions. The developed sensors display high selectivity, long term stability, fast response and applicability over a range of pH and drug concentrations. Assay methods with these sensors require no prior treatment of the drug formulations, are simple, rapid, accurate, selective, costeffective and, thus, suitable for routine drug analysis and for quality control/quality assurance program in drug industry.

(A)



(B)



Scheme (1): Chemical structure of levofloxacin (A); and lomefloxacin (B).

Experimental

Apparatus

All potentiometric measurements were made at 25±1^{0}C with an Orion (Model 720) pH/ mV meter, in conjunction with Single junction Ag/AgCl reference electrode, Orion electrode (Model 90-01) filled with 10% (w/v) potassium chloride and levofloxacin or lomefloxacin PVC membrane sensor. Combination glass electrode, A Ross pH electrode (Orion Model 81-02) was used for all pH measurements.

Reagents and materials.

All chemicals were of analytical reagent grade unless otherwise stated and doubly distilled water was used throughout. Poly (vinyl chloride) powder (PVC), tetrahydrofuran (THF) were obtained from Fluka. Flavinic acid (Fl) and ammonium reinckate (Re) were purchased from Sigma Chem. Co. (St. Louis, MO., USA).

Pharmaceutical grade of the drugs were obtained from El Nasr Pharmaceutical chemicals Co. Pharmaceutical preparations containing levofloxacin or lomefloxacin were obtained from local drug stores. Levofloxacin or lomefloxacin (10^{-2}-10^{-6} M) standard solutions were prepared by appropriate dilution of the stock levofloxacin or lomefloxacin solution with 0.01 M acetate buffer (pH 5).

Preparation of ion pair complexes.

Levofloxacin or lomefloxacin reineckate and flavianate ion–pair complexes were prepared by slow addition of 20 ml of 10^{-2} M solution of ammonium reineckate, or flavinic acid reagents, respectively to 20 ml aliquots of 10^{-2} M aqueous levofloxacin or lomefloxacin hydrochloride solution. The mixtures were stirred for 10 min, filtered on Whatmann filter paper no., 42, washed with distilled water, dried at room temperature and ground to fine powder.

Preparation of poly (vinyl chloride) membranes

5 mg portion of levofloxacin or lomefloxacin ion-pair complex was thoroughly mixed in a glass Petri dish (5cm diameter) with 350 mg of 2-nitrophenyloctyl ether (*o*-NPOE) and 190 mg of poly (vinyl) chloride (PVC). The mixture was dissolved in a 5 ml of tetrahydrofuran (THF).[27] The Petri dish was covered, and left to stand over night to allow slow evaporation of the solvent at room temperature. A master PVC membrane (0.1mm thickness) was obtained.

Construction of membrane sensors

The PVC master membranes were sectioned with a cork porer (10 mm diameter) and glued to a poly ethylene tubing 2 cm length, 8 mm i.d) by using THF. A home made electrode body was used, which consisted of a glass tube, to which the poly ethylene tubing was attached to one end and filled with the internal reference solution (equal volumes of 10^{-2} M aqueous levofloxacin or lomefloxacin and 10^{-2} M KCl). An Ag/AgCl internal reference wire electrode (1.0 mm diameter) was immersed in the internal solution. The electrodes were conditioned by soaking in 10^{-2} M levofloxacin or lomefloxacin solution for 2h and stored in the same solution when not in use.

Sensor calibration

Aliquots (10 ml) of 10^{-1}-10^{-6} M aqueous levofloxacin or lomefloxacin in 0.01 M acetate buffer of pH 5 were transferred into 50 ml beakers. The levofloxacin or lomefloxacin PVC membrane sensor in conjunction with the single junction Ag/AgCl reference electrode was immersed in levofloxacin or lomefloxacin solution. The solution was stirred and the potential reading was recorded after stabilization to ±0.2 mV. The e.m.f was plotted as a function of log (levofloxacin or lomefloxacin concentration). Alternatively, the levofloxacin or lomefloxacin PVC membrane sensor in conjunction with the single junction Ag/AgCl reference electrode was immersed in a 50 ml beaker containing 10 ml of 0.01 M acetate buffer of pH 5. Aliquots (1.0 ml) of 10^{-5}-10^{-1} M standard levofloxacin or lomefloxacin solution were successively added and the potentials were recorded after stabilization to ±0.2 mV after each addition. The e.m.f was plotted as a function of log (levofloxacin or lomefloxacin concentration). The calibration plot was used for measuring samples of unknown concentration under the same conditions.

Effect of pH on the sensors response

The effect of pH on the potential readings of the electrode systems was studied by immersing the Ross combination glass electrode, PVC levofloxacin or lomefloxacin sensors and a single junction Ag/AgCl reference electrode in 100 ml beakers containing 30 ml aliquots of 10^{-3} and 10^{-4} M levofloxacin or lomefloxacin aqueous solutions. The pH of each solution was gradually changed by adding small aliquots of dilute sodium hydroxide and/or hydrochloric acid solutions. The potential reading at each pH value was recorded.

Sensor selectivity

The potentiometric selectivity coefficients ($K^{Pot}_{I,J}$) of the levofloxacin or lomefloxacin sensors were measured by the separate solutions method.[28-30] In this method, the potentials of 10^{-3} M concentration of both lomefloxacin and the

interfering species in 0.01 M acetate buffer of pH 5 were determined. The selectivity coefficients were calculated using the following equation:

$$-\text{Log}\left(K_{L,I}^{Pot}\right)=(E_L-E_I)/S-[1-Z_L/Z_I]\ \text{Log}\ [L]$$

Where E_1 and E_2 are the potential readings observed after 1 min of exposing the sensors to the same concentration of levofloxacin or lomefloxacin and interferents, Z_L and Z_I are the charges of levofloxacin or lomefloxacin and interfering species I, respectively, and S is the slope of levofloxacin or lomefloxacin calibration graph (mV/concentration decade).

Determination in pharmaceutical preparations.

The contents of five tablets were weighed and finally powdered in a small dish. An accurately weighed portion of the powder, equivalent to one tablet, was dissolved with 0.1M acetate buffer of pH 5 in a 100 ml calibration flask. A 3.0ml aliquot of the mixture was transferred to a 100ml calibration flask and diluted to the mark with 0.1 M acetate buffer of pH 5. The e.m.f of the solution was measured as described above and compared with the calibration plot. Alternatively, the standard addition technique was used by monitoring the potential of 50 ml drug test solution before and after addition of 0.5ml of standard 10^{-2} M lomefloxacin solution.

Results and Discussion

The present work evaluates the possibility of using levofloxacin or lomefloxacin ion-pair complexes in the preparation of levofloxacin or lomefloxacin-selective electrodes in which PVC was used as polymeric matrix for immobilization of the sensors. The conventional design are prepared, characterized and compared according to IUPAC recommendations.[28]

Membrane composition and response.

Lomefloxacin or levofloxacin cation reacts readily with reineckate or flavianate anion to form 1:1 ion-pair complexes as confirmed by elemental analysis of the solid reaction products. Suitability and sensitivity of membranes, based on these ion-pair complexes as electroactive compounds were examined. The best membrane

composition for sensors based on levofloxacin or lomefloxacin–reineckate and levofloxacin or lomefloxacin-flavianate are 2:64:34 % (mg/mg) ion-pair complex; plasticizer and PVC, respectively. The plasticizer *o*-NPOE proved to be suitable plasticizer typical response curves of two lomefloxacin membrane sensors.

Table 1 summarizes the response characteristics of the levofloxacin and lomefloxacin membrane sensors, from data collected over a period of 4 months for 5 different membrane sensors of each type at pH 5 using both levofloxacin or lomefloxacin

Table (1): Response characteristics of levofloxacin (Le) and lomefloxacin (Lo) membrane sensors.

Parameter	Sensors			
	Le-Re	**Le-Fl**	**Lo-Re**	**Lo-Fl**
Slope, mV/decade	50.3±0.2	48.5±0.7	53.2±0.5	51.4±0.8
Correlation coefficient,(*r*)	0.998	0.997	0.999	0.998
Detection limit of linear range, (*M*)	10^{-4}-10^{-2}	10^{-4}-10^{-2}	10^{-4}-10^{-2}	10^{-4}-10^{-2}
Lower limit of detection, (*M*)	1×10^{-4}	1×10^{-4}	1×10^{-5}	1×10^{-5}
Working range, pH	4-6	4-6	4-6	4-6
Response time for 10^{-3} M, (*s*)	20±2	20±2	20±2	20±2
Recovery time for 10^{-3} M, (*s*)	40±2	40±2	40±2	40±2
Life span, (week)	4	4	4	4
Accuracy (%)	101.2	102.6	100.8	99.8
Repeatability, CV_w (%)	1.0	1.1	1.2	1.2
Between day-variability, CV_b (%)	1.1	1.3	1.2	1.3
Standard deviation, σ (%)	0.8	0.9	1.1	0.9

membrane sensors show near Nernstian slope 48.5-50.3 and of 51-53 mV/concentration decade over the concentration range 1×10^{-4}-1×10^{-2} M for levofloxacin and lomefloxacin membrane sensors respectively. The sensors displayed good stability at ambient temperature, potential reproducibility and reasonable selectivity.

The sensors showed rapid response within 20 seconds for drug solutions $\geq 10^{-3}$ M and 40 seconds for 10^{-4} M. The life–time of the sensors were examined by repeated calibration every 2 days. Over a period of 30 days, no noticeable deterioration in the sensor performance in terms of detection limit, calibration curve slope and response time was noticed.

Effect of pH and selectivity

The influence of pH of the test solution on the potential response of the membrane sensor was tested in the pH range 2-11. The pH was adjusted with dilute hydrochloric acid or sodium hydroxide solutions. The potentials remain constant from pH 4-6, beyond which the potential changes considerably.

The potentiometric selectivity coefficient of levofloxacin or lomefloxacin based

Table (2): Potentiometric selectivity coefficients of levofloxacin (Le) and lomefloxacin (Lo) membrane sensors.

Interfering ion (I)	Log ($K^{Pot}_{L,I}$)[a]			
	Le-Re	**Le-Fl**	**Lo-Re**	**Lo-Fl**
Co^{+2}	2.3×10^{-2}	1.1 ×10-1	1.1×10^{-1}	3.2×10^{-2}
Cs^{+}	4.0×10^{-3}	2.4×10^{-2}	2.4×10^{-2}	4.7×10^{-2}
K^{+}	7×10^{-3}	5.8×10^{-2}	7.8×10^{-2}	9.2×10^{-2}
Pb^{+2}	3.4×10^{-3}	1.0×10^{-2}	1.2×10^{-2}	2×10^{-2}
Mn^{+2}	2.1×10^{-2}	5×10^{-2}	7×10^{-2}	1.3×10^{-3}
Ni^{+2}	6.7×10^{-2}	6.9×10^{-2}	8.4 × 10-2	7×10^{-3}
Ca^{+2}	5.1×10^{-3}	4.0×10^{-2}	4.5 ×10-2	9×10^{-2}
Al^{+3}	3.9×10^{-2}	7×10^{-1}	6.6×10^{-1}	1×10^{-2}
Sr^{+2}	1.9×10^{-2}	4.9×10^{-1}	4.9×10^{-1}	2.5×10^{-1}
Cu^{+2}	5.1×10^{-2}	8.2×10^{-2}	8.7×10^{-2}	5.5×10^{-2}
Fe^{+2}	6.5×10^{-2}	8.8×10^{-1}	9.8×10^{-1}	2.6×10^{-1}
Ciprofloxacin	2.3×10^{-1}	2.1×10^{-1}	1.4×10^{-1}	1.6×10^{-1}

[a] Average of five measurements

sensors depends on the selectivity of the ion-exchange process at the membrane-sample interface, the mobility of the respective ions in the membrane, and the hydrophobic interactions between the primary ions and the organic membrane. Thus, the potentiometric selectivity of 10^{-3} M levofloxacin or lomefloxacin solution and 10^{-3} M of the foreign ion at pH 5 was critically investigated by the separate solution method. The influences of 12 different organic and in organic cations on the response of levofloxacin or lomefloxacin membranes were examined. The results obtained are

summarized in Table 2. The proposed sensors exhibited a high selectivity towards drugs with respect to the test ions.

Determination of drug

Levofloxacin or lomefloxacin in various drug formulations was determined by direct potentiometric measurements using both sensors of each drug. The potentials measured by these sensors were recorded and compared with data obtained using pure drug (not shown). Diluents and excipients normally used in tablet formulation did not show any interference. Thus, analysis was carried out without prior treatment or extraction. The developed procedure showed working characteristics adequate for determination of levofloxacin in pharmaceutical preparations. Table 3 shows the results obtained for the determination of levofloxacin in pharmaceutical preparations with average recoveries of 99.7% and 99.5%, and mean standard deviations were 0.3% and 0.5% (n=5) obtained for Le-Re and Le-Fl membrane sensors respectively.

Table (3): Determination of Levofloxacin in pharmaceutical formulations.

Pharmaceutical Preparations	Le Accuracy, [a] %		
	Le-Re	**Le- Fl**	**UV-Vis**
LEE- Flox Tablets (500mg) (Pharaoni pharmaceuticals)	99.7±0.2	99.5±0.7	97.3±0.5
Levoxin Tablet (250mg) (Amoun pharmaceuticals)	99.5±0.5	99.6±0.5	95.2±0.7
Levanic Tablet (500mg) (Medical union pharmaceutical)	99.9±0.4	99.5±0.3	96.6±0.1

[a] Average of five measurements

Table 4 shows the results obtained for the determination of lomefloxacin in pharmaceutical preparations with average recoveries of 99.3% and mean standard deviation 0.5% (n=5) was obtained for both Lo-Re and Lo-Fl membrane sensors respectively. The data were compared with that obtained using UV-Vis method.[19] Also the results obtained using spiking technique, proved the sensitivity and applicability of drugs membrane sensors.

Table (4): Determination of Lomefloxacin in pharmaceutical formulations.

Pharmaceutical Preparation	Lo Accuracy, [a] %		
	Lo-Re	Lo- Fl	Uv-Vis
Lomoxen Tablets (400mg) (Minapharm A.R.E. Egypt)	99.1 ±0.2	99.0 ±0.2	97.8±1.0
Lomex Tablet (400mg) (Sigma pharmaceutical industries)	99.5 ±0.9	99.6 ±0.8	98.4±0.7
Lomeflox Tablet (400mg)	99.3 ±0.1	99.3 ±0.2	96.2±0.8

[a] Average of five measurements

Validation of the proposed potentiometric methods for determining Le and Lo were made by measuring the range (R), lower limit of detection (LOD), accuracy (recovery), precision (r), repeatability (CVw), between day-variability (CVb), linearity (correlation coefficient) and sensitivity (slope). Results obtained on five batches (five determinations each) using the quality assurance standards[31] are depicted in Table 1. These data support the application of the proposed methods for quality control assessment of drug formulations.

Conclusions

Potentiometric sensors based on the use of plasticized PVC matrix membranes incorporating Le-Re, Le-Fl and Lo-Re, Lo- Fl ion exchangers are prepared and used for quantitative determination of levofloxacin and lomefloxacin respectively. These sensors offer the advantages of fast response, reasonable selectivity, low cost. Electrochemical evaluation of these sensors reveals sub-Nernstian response with slopes of 50.3, 48.5 and 53.2, 51.4 mV/decade for Le-Re, Le-Fl and Lo-Re, Lo- Fl sensors respectively.

References

1. L. L. Shen; American Society for Microbiology, Washington D.C., 77 (1993).
2. D. C. Hooper, and J. S. Wolfson; American Society for Microbiology, Washington D.C., 53 (1993).
3. D. Croisier, M. Etienne, E. Bergoin, P. Charles, C. Lequeu, L. Piroth, H. Portier and P. Chavanet; Antimicrob. Agents Chemother., 48, 1699 (2004).
4. P.M. Roblin, M. R. Hammerschlag; Antimicrob. Agents Chemother., 47 1447 (2003).
5. M. A. Sabry; Mutagenesis, 23, 515 (2008).
6. W. I. Klimberg, E. C. Cox, L. C. Fowler, S. S. Kim and S. A. Callery D'amico; Urology, 51, 610 (1998).
7. Z. Dragica and S. Traje; J. Serb. Chem. Soc., 70, 1451 (2005)
8. G. C. Tozo Greci and N. R. Salgago Herida; J. AOAC International, 89, 1305 (2006).
9. M. Atef, F. Abdulkader; J. Pharm. Biomed. Anal., 40, 179 (2006).
10. X. Gao, G. Yao, N. Guo, F. An and X. Guo; Drug Discov. Ther., 1, 136 (2007).
11. M. A. Shibl, F. A. Tawfik, S. El-Houfy and J. F. Al-Shammary; J. Clin. Pharm. Ther., 16, 353 (1991).
12. M. Hernandez, F. Borrull, and M. Calull ; J. Chromatogr. B, 742, 255 (2000).
13. S. Hanwen, L. Liqing, and S. Ming; Chromatographia, 67, 399 (2008).
14. S. Hanwen, L. Liqing, and C. Xueyan; Anal. Sci. 2, 1145 (2006).
15. L. H. Nie, H. C. Zhao, X. Wang, L. Yi, Y. Lu, L. P. Jin, and H. M. Ma; Anal. Bioanal. Chem., 374, 1187 (2002).
16. Z. Nan, Z. Xiaoli and Z. Yunfang; Microchemical J., 75, 249 (2003).
17. S. Junfeng, S. Yong, G.Wei; Anal. Sci., 17, 1145 (2001).
18. G. G. Cristiani , S. Hérida, R. Nunes, Acta Farm Bonaerense, 24. , 24 , 406 (2005).
19. N. B. Suhagia, S. A. Shah, I. S. Rathod, H. M. Patel, Y. M. Rao, Indian J. Pharm., Sci., 68, 247 (2006).
20. A. S. Amin, H. A. Dessouki and I. A. A.gwa; Arabian J. Chem., 1, 209 (2008).
21. Y. M. Issa, F. M. Abdel-Gawad, M. A. A. Table, and H. M. Hussein; Anal. Lett., 30, 2071 (1997).
22. A. M. El-Brashy, M. E. Metwally, and F. A. El-Sepai; Bull. Korean Chem. Soc., 25,

365 (2004).

23. G. L. Prabhushankar, B. Gopalkrishna, M. K. Manjunatha and H.G. Girisha: Int. J. Pharmc. Pharm. Sci., 2, 162 (2010).
24. S. Ulu, Eur. J. Med. Chem., 44, 3402 (2009).
25. K. Kaur, B. Singh and A. K. Malik; Thai J. Pharm. Sci. 34, 58 (2010).
26. H. Salem; American J. App. Sci., 2, 719 (2005).
27. Y. M. Issa, S. S. Badawy, A. A. Mutair; Anal. Sci., 21, 1443 (2005).
28. IUPAC Analytical Chemistry Division Commission on Analytical Nomenclature, Pure Appl. Chem., 72, 1851 (2000).
29. S. S. M. Hassan and N. M. H. Rizk; Anal. Letters, 33, 1037 (2000).
30. S. S. M. Hassan, Eman M. Elnemma, W. H. Mahmoud, A. H. K. Mohammed, J. App. Electrochem., 36, 139 (2006).
31. J. K. Taylor, Quality Assurance of Chemical Measurements (CRC Press, Florida, 1987).