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Potentiometric and Spectrofluorimetric Studies on Complexation of Levofloxacin with some Metal Ions

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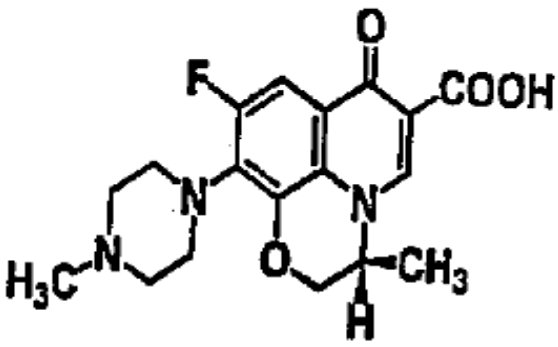
Summary. The interaction of levofloxacin with six metal ions: A1(III)., Cr(III), Sb(III) Bi(III), Fe(III) and Zr(IV), was studied using potentiometric and fluorimetric techniques. In the potentiometric study, the ionization constants of the ligand and stability constants of the complexes formed have been determined at 25± 0.1°C, ionic strength of NaN03 was 0.05 mol dm^{-1} . Complexes were formed by molar ratio metal to ligand, of 1:1, 1:2 and/or 1:3. The drug has a native fluorescence in DMSO which can be measured at $\gamma cm.$ 490 nm and γcm 297 nm. The presence of some metal ions increases the fluorescence intensity, while the presence of zirconium ion decreases the fluorescence intensity. The linear ranges of the compound and its chelates were 0.070 - 0.700 and 0.025-0.900 jig ml'1 in the absence and presence of different metal ions, respectively. Levofloxacin was determined by the proposed procedures in tablets. The recovery percent ranged from 99.94 to 101.19%. These procedures were also applied for the determination of drug in urine samples with an average recovery of 98.99 to 102.40%. The effect of other fluoroquinolones and the degradation product (decarboxylated levofloxacin) on the fluorescence intensity of levofloxacin was investigated.

Introduction

Levofloxacin [S-(-)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-l-piperaz-inyl)-7-oxo-7H-pyrido[l,2,3-de]-l,4-benzoxazme-6-carboxylic acid] has a broad spectrum antimicrobial activity on gram positive and gram negative bacteria.

Levofloxacin is generally considered to be about twice as active as its isoraer, ofloxacin. It is given by mouth or intravenous for the treatment of susceptible infections and also used topically for the treatment of bacterial conjunctivitis/1* The drug undergoes limited metabolism, it is mainly eliminated unchanged; within 24 hours of oral administration, intact drug is recovered in the urine (80- 85%)



Levofloxacin

Several methods have been published for determination of levofloxacin. These methods include HPLC, flow injection analysis, pulse polarography and, adsorptive square wave anodic stripping voltammetry technique. (ISJ Infrared spectrometry and NMR spectroscopy were also used. Both IR and NMR were used for comparison of the complication of some fluoroquinolones with certain metal ions. Spectrophotometric method was used for determination of levofloxacin by formation of binary complex with xanthene dyes. Spectrofluorimetric technique was used for determination of levofloxacin through either native fluorescence or through charge transfer complex formation with 7,7,8,8-tetracyanoquinodimethane (TCNQ). Synchronization-1sl derivative fluorescence spectroscopy was used for determination of levofloxacin enantiomers.

The present work describes potentiometric and Spectrofluorimetric studies on the complication of levofloxacin with certain metal ions. The Spectrofluorimetric method was successfully used for the rapid determination of levofloxacin in both tablets and urine.

Experimental

Potentiometric measurements

Reagents

Levofloxacin was obtained from Sinochem Hebei (China) and used as received; its solution was prepared in pure double distilled water. The metal ions used; A1(H1), Cr(III), Sb(III), Bi(III), Fe(III), and Zr(IV) were prepared as metal nitrate (BDH or E-Merck). Sodium nitrate (AR) solution was used for adjusting the ionic strength.

Apparatus

Measurements of pH were carried out on a pH meter model 3305 Jenway Ltd, (UK). Calvin-Bjerrums technique as adopted by Irving and Rossotti was used to determine the profanation constant of the ligand and the formation constants of the metal complexes at 25°C±0.1 in methanol (10% v/v) aqueous solution. During the titration oxygen free nitrogen was bubbled through the solution. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities. Thus, all constants determined in this work are concentration constants.

procedures

The following solutions were prepared and titrated potentiometrically against standard carbonate free-sodium hydroxide 0.1 mol dm"3 solution (standardized against standard potassium hydrogen phethalate):

a) 5 ml of 0.1 mol dm^{-3} HNO_3
b) a + 10 ml of 0.05 mol dm^{-3} ligand
c) b + 2.5 ml Of 0.1 mol dm"3 metal

Five milliliter of methanol was added and the total volume was adjusted to 50 cm3 by

water.

Spectrofluormetric measurements

Reagents

Commercial dosage forms of levofloxacin, Tavanic tablets (Hochest Marion Roussel Egypt) labeled to contain 250 mg of levofloxacin, Norfloxacin (Egyptian international pharmaceutical industrial Co., Cairo, Egypt), ofloxacin (Daiichi, Tokyo, Japan), ciprofloxacin (Miles Inc. Pharmaceutical Division, West Haren, Germany), lomefloxacin hydrochloride (Seavel, I liniois, USA). All other reagents and solvents were used of analytical grade. Human urine was kindly provided from healthy volunteer.

Apparatus

Shimadzu RF-5301 PC (Tokyo, Japan) Spectrofluorometer with 1-cm quartz cells was used for all measurements. The slit width of both excitation and emission monochromators was set at 3 nm. Super-mixer (Lab-line Instruments, Inc., USA) water bath (Schetzort, Germany).

Standard drug solutions

An accurately weighed amount (10 mg) levofloxacin was transferred into 100-m] calibrated flask and dissolved in about 10 ml of double distilled water (or in methylene chloride for determination in urine sample). The contents of the flask were completed to the mark with water (or methylene chloride) to provide standard solution containing 100 fig ml^{-1} of levofloxacin. From this solution, serial dilutions were made to obtain] concentrations in the range 0.2-9.0 μg ml^{-1}.

Preparation of sample

Analysis of tablets

Twenty tablets were accurately weighed and finely powdered. An amount of powdered tablet equivalent to 10 mg of levofloxacin was transferred to a 100-mI calibrated flask and dissolved in 40 ml water. The mixture was sonicated for few minutes and then completed to the mark with water. The solution was filtered and the first portion of the filtrate was discarded. Serial dilutions of this filtrate were made .to obtain the suitable concentration.

Analysis of urine samples

Into a test tube, one milliliter of standard solution of the drug in methylene chloride (0.2-0.7 μg ml^{-1}) was pippitted and evaporated till dryness on a water bath. One milliliter of urine sample was added and shacked well on a vortex. The spiked urine was extracted twice with methylene chloride each of 4 ml and the total extract was collected into a 10-ml calibrated flask and evaporated till dryness on a water bath. The flask was cooled and the procedure was completed as under the general assay procedures.

General assay Procedures

One-milliliter aliquot volume of either of standard, sample or degradation product solutions. was transferred into a 10-ml calibrated flask and completed with dimethylsulphoxide (DMSO) for native fluorescence or 0.5-1.5 ml of the metal solution was added. Volume was completed with the suitable solvent, see table 2. The fluorescence intensity was measured at γcm 490 nm. (γcm 297 nm). The prepared solutions remain stable for at least 24 hours.

Molar ratio method of Yoe and Jones

Equimolar solutions (3x 10^{-5}M) of levofloxacin and metal ions as nitrate were prepared. One milliliter of each metal ion solution was transferred into each of seventeen 10-ml calibrated flasks. Different volumes (0.5, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0,2-25, 2.50, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25 or 4.5) ml of levofloxacin solutions were added. These solutions were completed as under general procedures using suitable solvent for each metal. The fluorescence intensity of these solutions was measured at 490 nm (excitation at 297 nm) against blank treated similarly.

Results and discussion

potentiometric study

Proton-ligand systems

The constructed pH metric titration curves for free and metal complexes are shown in (Fig. 1). The acid dissociation constant of levofloxacin in 10% (v/v) methanol-water has been determined from curves a and b using a computer program based on the Irving-Rossotti program. The SUPERQUAD computer program was used

to refine the protonation constants of the drug. Levofloxacin possesses two ionizable functional groups a carboxylic group (pK_a = 6.3) and a basic piperazinyle group (pK_a= 8.3). These results show high degree of agreement with the reported values (pK_a = 6.05 (29}and pK_a = 8.22 (Table1).

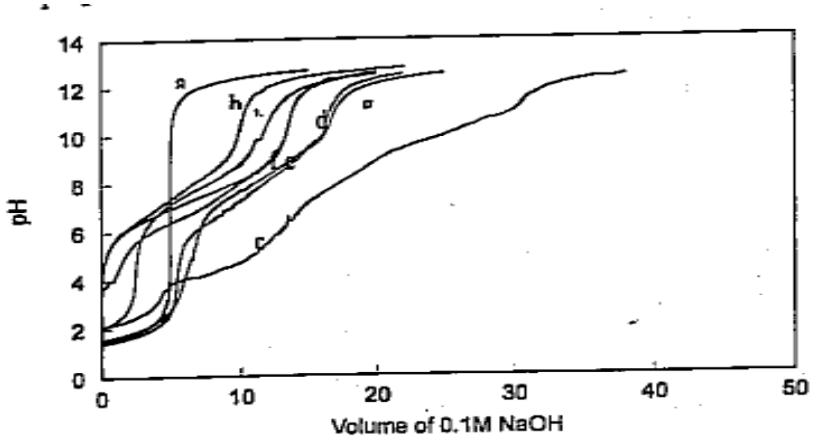


Fig. 1: Potentiometric titration curves of (a) 0.01 M nitric acid, (b) a + 0.02 M levofloxacin, (c) b + 0.01 M Al (III), (d) b + 0.01 M Cr (III), (e) b + 0.01 M Sb (III), (f) b + 0.01 M Bi (III), (g) b + 0.01 M Fe (III) and (h) b + 0.01 M Zr (IV) with 0.01 M sodium hydroxide.

Table 1. Formation constant of levofloxacin and stability constants of Metal ion complexes.

Central ion	Log K1	Log K2	Log K3
IT	8.30	6.30	-
Bi(III)	12.00	3.00	-
AL(II)	7.90	4.70	3.70
Cr(II)	4.00	2.55	-
Fe(II)	8,80	2.45	-
Sb(II)	2.85	2.54	-
zr(iv)	2.16	2.50	-

Temp. 25°C, μ = 0.05 mol dm^{-3} (NaN03) and 10% (v/v) methanolic solution.

Metal-Ligand systems

Analysis of curves c-h (Fig. 1) shows that the addition of metal ions to the solutions of the free ligand shifts the buffer region of the ligand to lower pH values indicating that the complication reactions proceed by releasing of protons. The values of stability constant of

levofloxacin complexes with metal ions (Table 1) were computed using standard procedures based on the calculation of the average number of ligands bonded per metal ions n' and the free ligand exponent, pL, as described previously .All studied metal ions form 1:1 and 1:2 metal/ligand complexes, while AI(III) forms 1:1, 1:2 and 1:3 complexes. Previous report indicated that A1(III) forms with levofloxacin complexes in the ratios 1:1,1:2 and 1:3.

Spectrofluorimetric measurements

Levofloxacin has a weak native fluorescence in aqueous solution that can be measured at 490 nm (excitation at 297 nm) (Fig. 2).

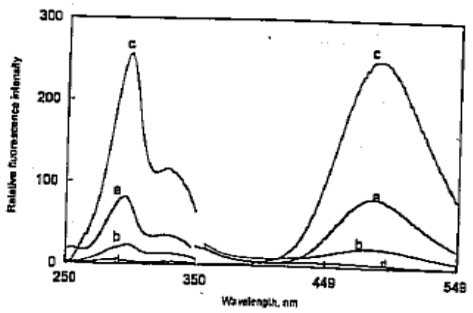


fig. 2: Excitation and emission spectra of (a) pure levofloxacin, (b) degradation products, (c) pure levofloxacin with Fe(IH) and (d) degradation products with Fe(in). (400 rag ml"1).

Table 2. Effect of diluting solvent on the Fluorescence of levofloxacin and its chelates with different metals.

Solvent	**Relative fluorescence intensity at γ_m 490 nm**						
Levofloxacine				**L-metal chelates**			
		A1(III)	**Cr(III)**	**Sb(III)**	**Bi(III)**	**Fe(III)**	**Zr(IV)**
Water	123	141	156.7	142	154	129 264	13
MeOH	83	9	287	293	283	340	299
EtOH	72	78	361	325	352	31	90
DMF	-ve	1	16	287	5	244 294	3
DMSO	233	213	255	233	261		245
CH_2CN	24	15	347	-ve	250		308

Optimization of variables Effect of solvent

The influence of different solvents on the fluorescence intensity, of the drug has been investigated. Table 2 shows that levofloxacin in dimethylformamide has no fluorescence, while its solution in ethanol, methanol, acetonitril and water shows weak fluorescence. High fluorescence intensity was obtained using dimethylsulphoxide and this may be due to its good solvating capacity for the fluorophore. The effect of different solvents on the fluorescence intensities of different metal-drug chelates was also studied. Table 2 shows that DMSO is the most suitable solvent for chelate formation of drug with A1(IU), while acetonitril gives the highest intensity in case of the chelate with Zr(IV). Ethanol was selected for chalets from drug with each of Bi(III), Cr(III), Fe(III) and Sb(III).

Effect of metal ions concentration

The effect of the addition of several metal ions on the fluorescence intensity of levofloxacin was also investigated using six metal ions Al(III), Cr(III), Sb(III), Bi(III), Fe(III) and Zr(IV). It was noted that the addition of any of these metal ions has no effect on the excitation or emission maxima, while their presence enhance the intensity and thus increase the sensitivity of the method. The optimal concentrations and volumes of these metals are shown in Table 3.

Table 3. Optimal conditions for fluoriraetric determination of levofloxacin in absence and presence of metal ions.

	Pure	**Al (III)**	**Cr(III)**	**Sb(III)**	**Bi(III)**	**Fe(III)**	**Zr (IV)**
Conc. of metals (mg ml^{-1})		0.10	0.10	0.02	0.10	0.02	0.10
Volume of metal (ml)		0.5	1.5	0.5	1.5	0.5	0.7
Diluting solvent	DMSO	DMSO	EtOH	EtOH	EtOH	EtOH	MeCN

Molar ratio method of Yoe and jones:

The method was applied in order to study the stoichiometry of the reaction between levofloxacin and each metal ion studied. A plot of the relative fluorescence intensity at γcm 490 nm (γcm at 297 nm) of levofloxacin complex as a function of molar ratio (ligand/metal) was constructed. The plot consists of more than two portions intersecting at ligand to metal ratio equal to 1, 2 or 3. This

suggests the formation of complexes with a stoichiometric ratio of drug: metal equal to 1:1 and 2:1 for all metal "ions and me ratio 3:1 in case of A1(HI). These results show an agreement with the potentiometric study, Table 1.

Validation of the spectrofluoremetric methods

Linearity of the response.

Under the suggested experimental conditions, the linearity range was studied for levofloxacin alone or its complexes with the investigated metal ions. Table 4 shows the typical linear range, slope, intercept and correlation coefficient of the drug alone and the chelates with the studied metals, the drug alone can be determined in the range 0.07-0.70 fig ml^{-1} while in the presence of metal ions the effective range for all chalets ranged from 0.02 to 0.90 ug ml^{-1} with a good correlation coefficient in all cases.

Detection and quantitative limits

The detection limits were calculated as 3 σ /b where b is the slope and a is the standard deviation of the intercept. The quantitative limit was calculated as 10 σ /b. It can be seen from Table 4 that levofloxacin can be detected from 0.019 fig ml^{-1} in

absence of metal ions and from (0.004-0.022μg-ml^{-1}) in the presence of metal ions. The quantitative limit was 0.065μg ml^{-1} in the absence of the metal ions, while in the presence of the metal ions the quantitative limits ranged from 0.015 to 0.076 μg

ml^{-1}.

precision

It was assessed by performing replicate (n=6) analyses on three concentration levels (0.2, 0.4 and 0.7 μg ml^{-1}) for the drug alone or in the presence of different studied metal ions and calculating the relative standard deviation (RSD). It was found that for pure drug the RSD ranged from 0.955 to 3.740 while in the presence of different metal ions the RSD ranged from 0.383 to 2.540. The precision was also determined for the spiked urine samples (n=5) for drug alone with RSD ranged from 0.870 to 2.250 and in the presence of Fe(III) as a representative example with a RSD ranged between 1.340- 1.550.

Accuracy and recovery

Analysis of tablets

The developed spectrofluoremetric methods in the absence and presence of the studied metal ions were applied for the determination of levofloxacin in tablets; the

Obtained results of analysis are shown in Table 5. Good recoveries (99.99 to 101.20% ± SD ranged from 0.513 to 1.987) were obtained in all cases. The obtained results were compared to those obtained from a reported method. Good agreement between the suggested and reported methods appears from t and F values with respected to the theoretical values. This makes the suggested procedures suitable for determination of drug in tablets.

Table 4. quantitative parameters for the analysis of levofloxacin by the fluorimetric methods:

Drug with	Calibration Range (ng ml^{-1})	Intercept ± SD	Slope ± SD	Correlation Coefficient	Determination Coefficient	LOD (ng ml^{-1})	LOQ (ng ml^{-1})
Pure	70 –700	6.6887 ± 2.9651	0.4522 ± 0.0075	0.9991	0.9983	19.67	65.57
Aluminum	20 – 700	1.4120 ± 0.6413	0.4245 ± 0.0073	0.9999	0.9999	4.53	15.10
Chromium	40 – 800	5.7555 ± 2.3420	0.7107 ± 0.0048	0.9998	0.9990	9.88	32.95
Antimony	25 – 500	1.9015 ± 1.5663	0.6478 ± 0.0053	0.9999	0.9998	7.25	24.18
Bismuth	65 – 700	14.0269 ± 0.0075	0.6772 ± 0.0108	0.9993	0.9987	20.14	63.11
Ferric	20 – 600	1.1595 ± 1.2534	0.6784 ± 0.0039	0.9999	0.9998	5.63	18.79
Zirchonium	80 – 900	38.6646 ± 4.1099	0.5392 ± 0.0063	0.9997	0.9994	22.86	76.23

Table 5. Analytical recovery of levofloxacin from Tavanic tablet (250 mg).

Form of drug	%Recovery ± S.D.	Form of drug	% Recovery ± S.D.
Pure drug	100.1 ± 0.513 t = 1.051 F = 3.751	Complex with Bi(III)	101.2± 1.987 t = 0.406 F = 1.871
Complex with Al(III)	100.9 ± 0.958 t = 0.246 F = 2.301	Complex with Fe(III)	100.9 ± 0.947 t = 0.089 F = 2.353
Complex with Cr(III)	100.5 ± 1.289 t = 0.344 F = 1.269	Complex with Zr(IV)	100.9 ± 0.713 t = 0.315 F = 4.157
Complex with Sb(III)	99.9 ± 0.844 t = 1.232 F = 2.962		

Theoretical values of F and t at 95% confidence limit are 5.05 and 2.228.

Average of six determinations

Reported methods , recover ± S.D. = 100.0± 1.418% (n=6)

Analysis of spiked urine

Urine samples were spiked with three concentrations of levofloxacin, 0.2, 0.5 and 0.7 μg ml^{-1} and extracted as mentioned under the procedure. The recoveries ranged from 102:1 to 102.4% (n = 5) with a RSD ranged from 0.87 to 2.25 in absences of metal ions. "While in presence of Fe (III) as a representative example the recoveries ranged from 98.99 to 100.76 (n =5) with RSD ranged from 1.34 to 1.55 (Table 6). This indicates that the presence of metal ions improves the recovery and decreases the interference, so the suggested methods are suitable for determination of levofloxacin in urine.

Table 6. recoveries of levofloxacin from urine by the fluorimetric method in absence and presence of Fe (III).

Spiked levofloxacin μg ml^{-1}	% recovery ± SD of levofloxacin	% recovery ± SD of levofloxacin with Fe (III)
0.2	102.4±0.87	100.76±1.44
0.5	102.2±2.15	100.32±1.55
0.7	102.1±2.25	98.99±1.34

Interference

The effect of other fluoroquinolones on the relative fluorescence intensity of levofloxacin was investigated. ofloxacin, the isomer of levofloxacin gave the same response. Norfloxacin, ciprofloxacin and lomefloxacin had no effect on the relative fluorescence intensity (RFI) of levofloxacin. This difference in behavior may be due to the presence of benzoxacine ring in levofloxacin which hold the structure in rigid form giving its native fluorescence. While, in other compounds, norfloxacin, ciprofloxacin and lomefloxacin this moiety is absent, so they haven't native fluorescence and so haven't any interference effect on levofloxacin. The main degradation products of fluoroquinolones are their decarboxylated compounds. The decarboxylated levofloxacin, has a little effect on the relative fluorescence intensity of intact levofloxacin and the presence of Fe(III) as a representative example decreases this effect (Fig. 2).

In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by determining synthetic mixtures of levofloxacin in the presence of its degradation product (decarboxylated levofloxacin at different concentration levels in absence and presence of Fe(HQ. It was noted that analysis of mixture of Jevofloxacin and its decarboxylated compound in the absence of Fe(III) revealed that decarboxylated compound does not interfere with the determination of the intact drug up to 75 % in the lower concentration (0.2 μg ml^{-1}) [recovery % = 99.81-103.77, n=4]. With a middle concentration (0.4 μg ml^{-1}) the degradation product does nofinterfere till 50% [recovery % = 99.09-105.66, n= 4]. While, at higher concentration (0.7 μg ml^{-1}) it interfered with the determination of the intact drug at any percent. When the percentage of the degradation product increases, the recovery percent also increases. However, addition of Fe(III) leads to improvement of the recovery percent (Table 7) till to the percent of 75% of the intact drug at concentration levels [recovery % = 98.83-103.02]. When the percentage of the degradation product increase than 75% of the intact drug the recovery percent also increases. The results obtained indicated that presence of Fe(III) increases sensitivity and reduces the interference from the degradation product so it can be used as stability indicating assay.

Table 7. Recovery of levofloxacin in presence of its degradation product (decarboxylated levofloxacin).

Conc. of drug (fig ml'1)	Cone, of degradation Product (fig ml"1)	% Recovery ± SD	
		Presence of Fe (HI)	Absence of Fe (HI)
0.2	0.050	99.81 ±0.59	99.15 ±0.99
	0.100	103.77 ±3.83	100.39 ±0.77
	0.150	101.26±2.29	101.19± 0.49
	0.200	141.43 ±3.09	109.40 ±0.56
0.4	0.100	99.05 ±1.96	103.54±1.21
	0.200	105.66 ±3.96	99.03 ±1.61
	0.300	134.98 ±1.15	99.94 ±0.97
	0.400	152.49 ±3.39	122.89 ±2.26
0.7	0.175	107.29 ±1.29	101.62 ±1.99
	0.350	160.93 ±7.19	98.83 ± 2.45
	0.525	218.86 ±9.49	103.02 ±0.19
	0.700	255.13 ±15.27	123.94 ±2.72

Conclusion

On the basis of potentiometric measurements, the complex formation between levofloxacin and metal ions was studied. Both the ionization constants of levofloxacin and stability constants of the formed complexes were evaluated. Simple and rapid methods for spectrofluoremetric determination of levofloxacin both in absence and presence of certain metal ions were developed. The methods were successfully applied for the determination of drug in tablets and urine. The method (in presence of metal ions) can be used as stability indicating assay for the determination of drug in the presence of its degradation product (decarboxylated levofloxacin).

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