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Simple Potentiometric Study for the Detection of Levofloxacin Hydrochloride and Daclatasvir Dihydrochloride in Pure form and Pharmaceutical Preparations

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ABSTRACT

The present study aimed to suggest a sensitive, precise and selective electrochemical approach for the potentiometric detection of Levofloxacin hydrochloride (LVX) and Daclatasvir dihydrochloride (DAC). The suggested technique was conducted by the incorporation of LVX or DAC with precipitating agent to construct two active sensors. Different concentration ranges of the investigated drugs ($1.0x10^{-5}$-$1.0x10^{-2}$ and $1.0x10^{-6}$-$1.0x10^{-3}$ mol L^{-1}) were detected under optimum conditions and provided potentiometric responses of 58.7±0.2 and 28.7±0.5 mV $decade^{-1}$ with detection limits $3.2x10^{-6}$ and $1.1x10^{-7}$ for the two fabricated sensors, respectively. The fabricated LVX-TPB and DAC-TPB were successfully used for excellent detection of pure form and tablets of LVX and DAC.

Keywords: Levofloxacin hydrochloride, Daclatasvir dihydrochloride, Plastic membrane sensors, potentiometric approach, Pharmaceutical dosage forms.

INTRODUCTION

The use of electrochemical sensors has explored as a new class of chemical read-outs for monitoring a variety of chemical species because of their unique physico-chemical features and sensing properties. Promising applications have been found by potentiometric approach in various pharmaceutical analysis[1-3], diverse chemicals of food quality[4], clinical and biological interest[5,6]. Various analytical benefits of potentiometric sensors, including their high stability and electrical conductivity have been reported[7].

Levofloxacin hydrochloride (LVX) is a member of the family of medications known as

broad spectrum antibiotics. It is commonly recommended in urinary tract infections, gastrointestinal tract. Also, it is prescribed to treat the respiratory tract infection and pelvic inflammatory diseases[8]. LVX (Fig. 1a) is a member of quinolones, and its literature survey addressed some analytical techniques for the determination of LVX in different matrices. Among these methods are spectroscopic methods such as spectrophotometry[9-15]. Furthermore, LVX was detected using high performance liquid chromatography[16-19].

Daclatasvir dihydrochloride (DAC) (Fig. 1b), is recommended for the treatment of hepatitis C virus by inhibiting NS5A protein. It is a new oral antiviral medication which exhibits a potential pangenotypic activity[20, 21]. Few chromatographic methods were reported, including high performance liquid chromatography [22-28]. The investigated drug was detected using spectrophotometric method[29,30]. A chitosan modified electrochemical electrode for the detection of DAC was also, reported [31].

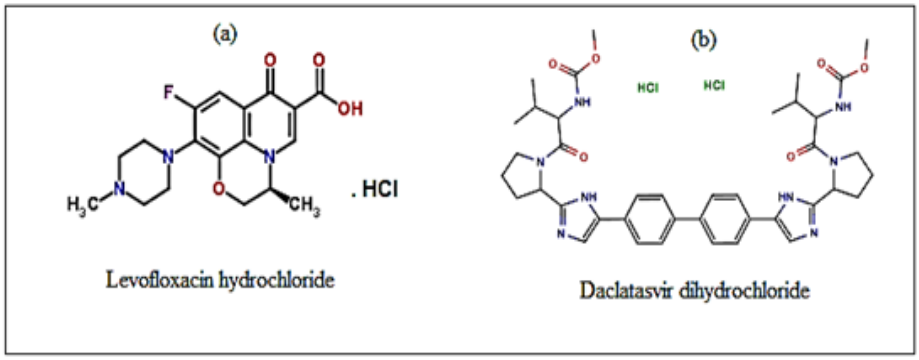

Fig. 1. Chemical structures of (a): Levofloxacin hydrochloride and (b): Daclatasvir dihydrochloride

The present study aimed to suggest two simple and accurate TBP-LVX and TPB-DAC plastic membrane sensors for LVX and DAC determination in their bulk powder and commercial formulations.

EXPERIMENTAL

Chemicals and reagents

Pure grade LVX and DAC were provided by Pharmaceutical Co. (Memphise). Daclenza® (60 mg/tablet) and Unibiotic® (500 mg/tablet) were purchased from local drug stores. Sodium tetraphenyl borate (TPB) purity of (99.5 %), dioctylphthalate (DOP, 98.0 %), Tetrahydrofuran (THF), hydrochloric acid 36%, methanol, Polyvinyl chloride (PVC), microcrystalline cellulose, L-histidine, L-cysteine, titanium dioxide, talc, starch, mannitol, lactose and magnesium stearate, were acquired from (Sigma-Aldrich, Hamburg, Germany). Sodium hydroxide, potassium chloride, sodium chloride, calcium carbonate, barium chloride and copper sulfate were supplied by (BDH laboratory supplies, Poole, UK).

Instrumentation

The potentiometric detections were performed under continuous magnetic stirring at 25±1°C with a Jenway pH-meter. A saturated standard electrode; silver/silver chloride double junction electrode was used. The same model of pH meter was used for pH adjustment.

Preparation of analytical solutions

Stock LVX and DAC

A solution of each LVX and DAC was daily prepared by dissolving 1.98 g and 4.05 g in 50 mL water forming a concentration of 0.1 mol L^{-1}. Modelling analytes in the range of 1.0x 10^{-7}-1.0x 10^{-1} mol L^{-1} was daily obtained.

LVX and DAC tablet solutions

Not less than 10 tablets of each Daclenza® (60 mg/tablet) and Unibiotic® (500 mg/tablet) were finely agitated and 0.01 mol L^{-1} tablet solution was formed from each LVX and DAC into 10 mL methanol. A clear solution was taken and completed to volume using distilled water, after complete centrifugation and filtration. Two ranges of working solution 1.0x10^{-5}- 1.0x10^{-2} and 1.0x10^{-6}-1.0x10^{-3} mol L^{-1} were produced by dilutions using ultra pure water for LVX and DAC detection, respectively.

Preparation of LVX-TPB and DAC-TPB ion pairs

The electroactive materials LVX-TPB and DAC-TPB of the developed sensors were prepared by the incorporation of equal volume of 0.01 mol L^{-1} of each LVX, DAC and TPB was mixed. Resulted precipitates were filtered and dried at room temperature overnight.

Sensor construction

Preparation of LVX-TPB and DAC-TPB plastic membrane sensors

The conventional PVC membrane sensors of LVX and DAC were fabricated by adding 190 mg PVC to 10.0 mg of each LVX-TPB or DAC-TPB ion pairs and 0.35 mL of DOP as plasticizer. Approximately, 5.0 mL of tetrahydrofuran (THF) was used. Membrane contents were poured into rounded glass dish and left aside for drying. The semi-transparent PVC membrane was obtained. The membrane is fitted with a polyethylene tube and the internal solution (1:1) 0.001 mol L^{-1} sodium chloride solution and LVX or DAC solution was used. Then, they were preconditioned in 0.001 mol L^{-1} LVX or DAC solution for 1 hours[32-34].

Sensor calibration

Calibration graphs of the developed LVX-TPB and DAC-TPB sensors were plotted using modelling solutions of $1.0x10^{-7}$-$1.0x10^{-1}$ mol L^{-1} LVX or DAC.

Standard addition method

The determination of each LVX and DAC in their commercial products was carried out using the standard addition method. It was conducted by dropping small additions to the analyte solution vs. the potential reading. The concentration of the test solution was obtained from (E_2-E_1).

RESULTS AND DISCUSSION

LVX and DAC sensors were fabricated using LVX-TPB or DAC-TPB electroactive materials. The sensitivity and selectivity of the constructed sensors were studied (Figure 2).

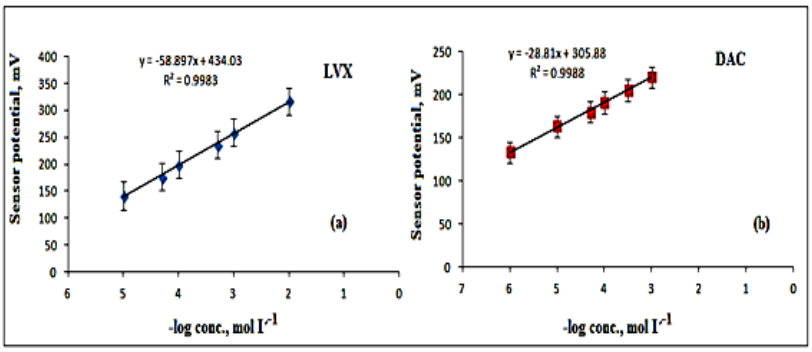

Figure 2: Typical calibration graphs of LVX-TPB and DAC-TPB plastic membrane sensors

Table 1: Critical analytical data of LVX-TPB & DAC-TPB PVCsensors

	LVX-TPB	DAC-TPB
Slope (mV decade^{-1})	58.8±0.3	28.8±0.6
Correlation coefficient, r	0.9989	0.9995
Interccept	434.03	305.88
Linearity range (mol L^{-1})	$1.0x10^{-5}$-$1.0x10^{-2}$	$1.0x10^{-6}$-$1.0x10^{-3}$
LOD	$3.2x10^{-6}$	$1.1x10^{-7}$
LOQ	$9.7x10^{-6}$	$3.4x10^{-7}$
Response time/s	30	40
pH	3.5-5.5	4.5-7
Life time/day	40	35
Temperature °C	25 °C	25 °C
Accuracy (%)	99.7 ± 0.7	99.2 ± 0.4
Robbustness	99.2 ± 0.4	99.7 ± 0.3
Raggedness	98.6 ± 0.7	99.4 ± 0.1

It was found that LVX-TPB and DAC-TPB sensors were displayed Nernstian responses (58.8±0.3 and 28.8±0.6 mV decade^{-1}) covering 1.0x10^{-5}-1.0x10^{-2} and 1.0x10^{-6}-1.0x10^{-3} mol L^{-1}. The limits of detection 3.2x10^{-6} and 1.1x10^{-7} mol L^{-1} were recorded for LVX and DAC sensors. The quantification limits were also evaluated and was found to be 9.7x10^{-6} and 3.4x10^{-7} mol L^{-1} (Table 1).

The influence of three different kinds of plasticizers was carefully studied using DOS (ε= 4.0), DBS (ε= 4.5) and DOP (ε= 5.1). DOP was superior to other plasticizers in terms of sensor performance owing to higher (ε = 5.1) of DOP (Table 2).

Table 2. The resulted slopes of LVX-TPB and DAC-TBP PVS sensors using different plastecizers

	LVX-TPB	DAC-TPB
DOS	49.9	18.5
DBS	50.4	23.8
DOP	58.8*	28.8*

The investigated solutions in the range of 1.0x10^{-7}-1.0x10^{-1} mol L^{-1} was used to calculate the response time. Response times for LVX-TPB and DAC-TPB were 30 & 40 s for lifetime 40 & 35 days (Figure 3).

pH values for LVX-TPB and DAC-TPB sensors were evaluated using 1.0×10^{-3}, 1.0×10^{-4} mol L^{-1} and 1.0×10^{-4}, 1.0×10^{-5} mol L^{-1} solutions. The pH of the analyte solution was adjusted using dil. HCl. Then, dil. NaOH was added to gradually increase pH. The potentials were derived against pH. The constructed LVX-TPB and DAC-TPB were safely active in pH 3.5-5.5 and 4.5-7 (Fig. 4). The decrease in the pH below 3.5 and above 7 was attributed to the effect of H^+ or OH^-, respectively.

For investigating LVX-TPB and DAC-TPB selectivity coefficients, prepared sensors were employed to measure 1.0x10^{-3} mol L^{-1} of each drug in presence of different possible interfering species using SSM[35]. Fabricated sensors selectivity coefficients were calculated using the following equation.

$$\text{Log } K^{pot}_{drug}\, J^{z+} = (E_2 - E_1) / S$$

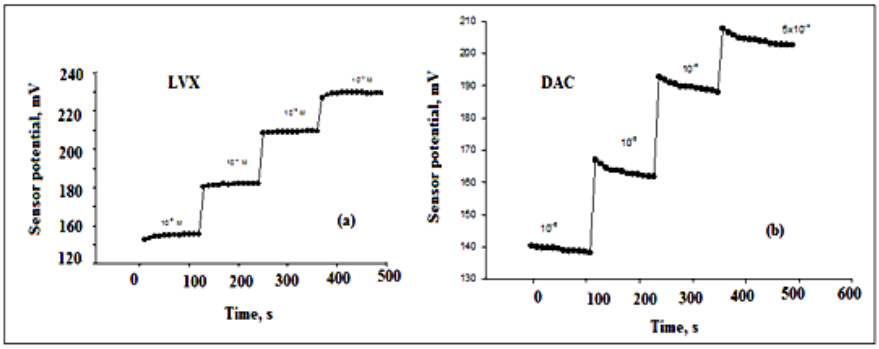

Fig. 3. Response time plot for (a) LVX-TPB and (b) DAC-TPB membrane sensors

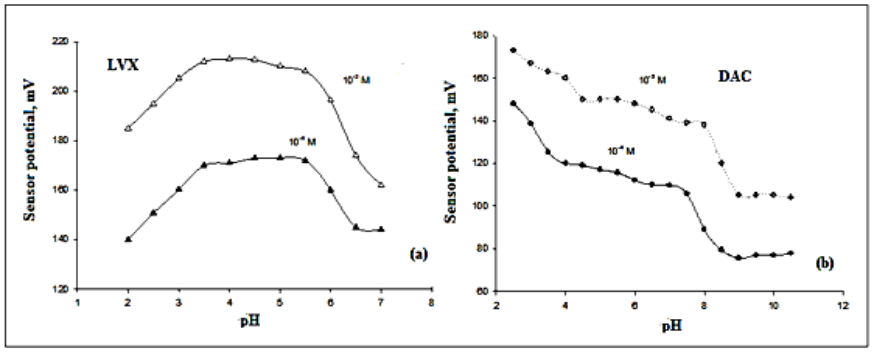

Fig. 4. pH influence on LVX-TPB and DAC-TPB sensors

No interference was noticed during the detection of LVX and DAC (Table 3).

Table 3: Selectivity coefficients ($K^{Pot}_{Drug^+}$) of LVX-TPB and DAC-TPB sensors

Interferent	$K^{Pot}_{PLZ^+}$ LVX-TPB plastic membrane sensor	DAC-TPB plastic membrane sensor
Na^+	$7.4x10^{-3}$	$1.1x10^{-3}$
K^+	$6.8x10^{-3}$	$7.6x10^{-4}$
Ca^{2+}	$1.5x10^{-4}$	$2.6x10^{-4}$
Ba^{2+}	$2.3x10^{-4}$	$5.3x10^{-4}$
Cu^{2+}	$3.5x10^{-4}$	$3.9x10^{-4}$
Lactose	$7.1x10^{-3}$	$1.2x10^{-3}$
Talc	$7.1x10^{-3}$	$1.0x10^{-3}$
Starch	$9.0x10^{-3}$	$2.8x10^{-3}$
Mannitol	$6.3x10^{-3}$	$3.9x10^{-3}$
L. Histidine	$2.5x10^{-3}$	$1.7x10^{-4}$
L. cycteine	$6.6x10^{-3}$	$2.7x10^{-4}$
Magnesium stearate	$5.6x10^{-3}$	$2.3x10^{-3}$
Titanium dioxide	$5.3x10^{-4}$	$8.9x10^{-5}$
Microcrystalline cellulose	$3.6x10^{-3}$	$4.2x10^{-4}$

Technique validation

It was conducted by evaluating various parameters in accordance with ICH guidelines[36].

The linear relationship of the suggested potentiometric method was evaluated using LVX and DAC test solutions of concentrations $1.0x10^{-7}$ to $1.0x10^{-1}$ mol L^{-1}. The two fabricated LVX-TPB and DAC-TPB sensors were employed for the detection of LVX and DAC test solutions. The results obtained revealed a concentration linearity of $1.0x10^{-5}$-$1.0x10^{-2}$, $1.0x10^{-6}$-$1.0x10^{-3}$ mol L^{-1} for LVX and DAC sensors, respectively.

Detection limits were evaluated when the slope was dropped by 17.9 mV. The results were $3.2x10^{-6}$ & $1.1x10^{-7}$ mol L^{-1}, while, quantification limits were $9.7x10^{-6}$ & 10^{-7} mol L^{-1} for LVX-TPB and DAC-TPB, respectively. Accuracy was examined in presenceof magnesium stearate using the standard addition method. The % recoveries were 99.7±0.7 & 99.2±0.4, for LVX-TPB and DAC-TPB sensors. Precision was evaluated using intra-day and inter-day assay. RSD was less than 1% indicating good precision.

Table 4: Data obtained by investigating the precision of the fabricated LVX-TPB and DAC-TPB sensors

Intra-day assay				Inter-day assay			
LVX-TPB		DAC-TPB		LVX-TPB		DAC-TPB	
Taken*	% Recovery	Taken	% Recovery	Taken	% Recovery	Taken	% Recovery
5.0	99.8±0.4	5.0	99.3±0.2	5.0	99.6±0.3	5.0	99.0±0.3
4.0	99.0±0.6	4.0	99.6±0.1	4.0	99.4±0.5	4.0	98.4±0.8
3.3	98.8±0.8	3.3	99.7±0.3	3.3	99.2±0.2	3.3	98.9±0.6

*- log concentration, mol L^{-1}

Robustness was tested by minor changes in pH at 5.5±1 and 7±1 for LVX and DAC respectively. Resulted data were 99.2±0.4 & 99.7±0.3 for LVX-TPB and DAC-TPB. Ruggedness was examined using (HANNA 211 pH meter). The obtained data were 98.6±0.7 & 99.4±0.1 for LVX and DAC.

Analytical applications

Quantification of LVX and DAC

LVX and DAC were quantified directly in bulk form using LVX-TPB or DAC-TPB sensors. The listed data were 99.3±0.4 and 99.5±0.5 for tested sensors (Table 5). Furthermore, LVX and DAC were estimated in their tablets; they were recovered by 99.3±0.6 and 99.4±0.5 (Table 6).

To evaluate the proposed method, the obtained results were statistically assessed using *t*- & *F*- tests at 95% confedence level [37]. The obtained

results for LVX detection using the fabricated LVX-TPB was compared with Maleque *el al.,* (2012) method in which LVX was detected using water: methanol: acetonitrile (9.0:5.0:0.5 v/v/v) as solvent at 292 nm[11]. Furthermore, the obtained results of DAC determination were compared with a simple spectrophotometric method which carried out by detecting the DAC drug in its bulk and pharmaceuticals, the detections were measured at 317 nm[30]. The outcome results revealed an excellent agreement with the previously mentioned published methods (Table 7).

Table 5: Data obtained by the detection of LVX and DAC in bulk drug using LVX-TPB and DAC-TPB sensors

Sample	LVX-TPB PVC sensor			DAC-TPB PVC sensor		
	Taken	Found	% Recovery	Taken	Found	% Recovery
Pure drug	5	4.99	99.8	6	5.97	99.5
	4.3	4.28	99.5	5	4.98	99.6
	4	3.97	99.3	4.3	4.24	98.6
	3.3	3.26	98.8	4	3.99	99.8
	3	2.98	99.3	3.3	3.29	99.7
	2	1.98	99	3	3	100
%Mean ±SD		99.3±0.4			99.5±0.5	
n		6			6	
Variance		0.16			0.25	
%SE		0.16			0.2	
% RSD		0.4			0.5	

Table 6: Data obtained by the detection of LVX and DAC in using LVX-TPB and DAC-TPB sensors using standard addition method

Sample	LVX-TPB PVC sensor			Sample	DAC-TPB PVC sensor		
Unibiotic® 500 mg/tablets	Taken	Found	% Recovery		Taken	Found	% Recovery
				Daclenza®			
	5	4.97	99.4	60 mg/ tablet	6	5.95	99.2
	4.3	4.26	99.1		5	4.93	98.6
	4	3.95	98.8		4.3	4.28	99.5
	3.3	3.3	100		4	3.99	99.7
	3	2.99	99.7		3.3	3.28	99.4
	2	1.97	98.5		3	3	100
% Mean ±SD		99.3±0.6				99.4±0.5	
n		6				6	
Variance		0.36				0.25	
% SE		0.24				0.2	
% RSD		0.6				0.5	

Table 7: Data obtained for the detection of LVX and DAC in their pharmaceutical formulations by the proposed and reported methods

Taken mol L^{-1}	Mean%±SD	n	Variance	%SE	%RSD	*t*-test	*F*-test	
LVX-TPB	$1.0x10^{-5}$-$1.0x10^{-2}$ $1.0x10^{-6}$-$1.0x10^{-3}$	99.3±0.6	6	0.36	0.24	0.6	0.83 (2.228)*	1.78 (5.05)*
DAC-TPB		99.4±0.5	6	0.25	0.2	0.5	0.581 (2.228)*	1.96 (5.05)*
Reported method of LVX [11]	1-12 µg mL^{-1}	99.7±0.8	6	0.64	0.32	0.8		
Reported method of DAC[30]	2-12 µg mL^{-1}	99.6±0.7	6	0.49	0.28	0.7		

* $t_{tabulted}$ and $F_{tabulted}$ [37]

CONCLUSION

This present study described a selective potentiometry approach for estimation of LVX and DAC in their bulk and pharmaceutical forms. Sensors were accurate and precise for the assay of LVX and DAC and displayed excellent detection of the investigated drugs with lower detection limits.

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